PRACTICAL CHEMICAL ANALYSIS OF BLOOD

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A BOOK DESIGNED AS A BRIEF SURVEY OF THIS SUBJECT FOR PHYSICIANS AND LABORATORY WORKERS

BY

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PREFACE

In 1920 the author published a series of articles in the *Journal of Laboratory and Clinical Medicine* under the title of "Chemical Changes in the Blood in Disease". A year later this material was revised and printed in book form as the first edition of Practical Chemical Analysis of Blood. The preface stated:

"The object has been to present briefly a discussion of the chemical blood determinations which have been found of definite value in the diagnosis and treatment of disease. Since 1912 the advances in this field have been very rapid, and there are now many practical tests with which the efficient internist and surgeon must be familiar. It is hoped that this little book may serve a useful function in indicating why, and how, certain chemical blood analyses should be made."

Many advances have been made in the field which this book is designed to cover since the publication of the first edition more than two years ago. This has made it necessary to enlarge and to alter slightly the general plan of the book. The first edition included only a single method for each determination at the end of the individual chapters. This arrangement was followed partly for the sake of brevity and partly because the methods described were especially suited to the scheme of blood analysis employed in the author's laboratory. This plan is continued in the present edition, but in addition it has seemed desirable to give the methods of the Folin-Wu system of blood analysis complete in a separate chapter (new Chapter IX). The method for uric acid given in Chapter III is the new and very simple method of Benedict.

A new Chapter X discusses various miscellaneous determinations not included in the main part of the book, and gives methods for such determinations as hemoglobin, oxygen capacity and content, calcium, inorganic phosphorus and acetone bodies. References are also given for other quantitative methods of blood analysis.

A number of practical questions which may arise in connection with the chemical analysis of blood for diagnostic purposes are discussed in Chapter XI on chemical blood analyses, their clinical use and interpretation. A few quantitative micro-methods of urine analysis of interest in connection with blood analyses are given in Chapter XII. The Appendix gives a rather full discussion of types of colorimeters and their use, an alphabetical list of the standard solutions and reagents employed in the various tests, tables of atomic weights and metric equivalents, and a four-place logarithm table to aid in calculations.

The author takes pleasure in acknowledging the aid of Dr. John A. Killian in collecting material for the preparation of this book. He is indebted to Miss Lela E. Booher for aid in the preparation of the manuscript. He also wishes to thank Dr. Stanley R. Benedict for permission to insert an unpublished method for the inorganic phosphorus.

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New York City.

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CHAPTER I

INTRODUCTION

ACCURATE data on the chemical composition of the blood, especially of the nonprotein fraction, are of comparatively recent origin, and are primarily the result of American observations with American methods. We owe largely to Folin, Benedict, and Van Slyke the development of the methods which have made this work possible. The practical information which these methods have made available has been especially helpful, since they have given us very valuable data on just those conditions on which the older methods of blood examination, cytology, bacteriology, serology, gave little information—reference is made principally to such constitutional conditions as nephritis, diabetes, and gout.

Although it is the primary object of this chapter to consider those observations which have served as a guide to the diagnosis and treatment of disease, it may be well at the outset to indicate the rather broad scope which chemical methods have recently taken in the study of the blood.[1] They have included methods for the blood volume, the blood proteins, serum albumin and globulin, improved methods of hemoglobin estimation; methods for the determination of the nonprotein nitrogen and its individual components, urea, creatinine, uric acid, amino acids, creatine and ammonia; methods for sugar, and for the lipoid constituents, fat, lecithin and cholesterol; methods for the mineral constituents, chlorides, phosphates, calcium, magnesium, potassium, sodium and iron; methods for the blood gases, carbon dioxide, carbon monoxide and oxygen, and methods for the hydrogen ion, the acetone bodies, phenol, and such enzymes as diastase, catalase, etc.

As already pointed out, these methods have yielded especially helpful information in diabetes, nephritis, and gout, while the data obtained in renal diabetes, infantile conditions such as tetany, rickets and the diarrheal acidoses, in eclampsia, pneumonia, malignancy, cholelithiasis, pernicious anemia, disorders of the ductless glands and various urologic conditions, have given us a new point of view regarding many of these disorders.

Before proceeding to a discussion of the pathologic variations in the composition of the blood, it may be well to indicate in tabular form the range of changes usually encountered in several common metabolic disorders. From an inspection of Table I it will be apparent that the tests furnish information of great value in diagnosis, prognosis, and treatment. Data are given on nine different blood constituents, the first five of which are regarded of special significance, and are arranged in order of their supposed clinical importance; viz., urea, sugar, CO_2-combining power, creatinine and uric acid.

TABLE I

SIGNIFICANT CHEMICAL CHANGES IN THE BLOOD IN DISEASE*

CONDITION	UREA N	SUGAR	CO_2-COMBINING POWER	CREATININE	URIC ACID	NON-PROTEIN N	CHOLESTEROL	CHLORIDES AS NaCl	DIASTATIC ACTIVITY
	mg. to 100	per cent	c.c. to 100	mg. to 100	mg. to 100	mg. to 100	per cent	per cent	
1. Normal	12–15	0.09–0.12	50–75	1–2	2–3	25–35	0.14-0.17	0.45–0.50	15–20
2. Beginning pathologic	20	0.15	–45	3.5	4	35	0.19	+0.52	25
3. Renal diabetes		0.08–0.12							
4. Mild diabetes		0.15–0.30							25–40
5. Severe diabetes	20	0.30–1.20	50–10	2–4	4–10		0.2-0.8	0.40	35–75
6. Gout					4–10				
7. Early interstitial nephritis	15–25	0.12–0.15		2–3.5	5–12			0.45–0.60	
8. Acute nephritis	40–100	0.12–0.18	45–20	2–6	5–15			0.45–0.60	
9. Parenchymatous nephritis (nephrosis)	20–50	0.12–0.20		2–4	2–5		high	0.50-0.61	
10. Terminal interstitial nephritis	60–300	0.12–0.24	40–12	5–28	5–27	100–350	to 0.30	0.36–0.60	20–50
11. Bichloride poisoning, to	300	0.12–0.20		33	15	370	0.35		
12. Double polycystic kidney, to	75	0.20		8	5				
13. Prostatic obstruction	12–40	0.11–0.16		1.5–3.5	3–9				
14. Acute intestinal obstruction	45–120			to 10		75–170			
15. Eclampsia	6–25	0.10–0.18	55–12		4–11	35–60		0.47–0.61	
16. Cholelithiasis							0.13–0.30		
17. Pernicious anemia							to 0.06		

*The data recorded in Table I are collected from analyses made in the author's laboratory, except for the figures given on intestinal obstruction. These are taken from data given by Tileston and Comfort,[2] and Cooke, Rodenbaugh and Whippel[3] on very severe cases. The figures under 11 and 12 (to) simply indicate the maximal values we have observed in these conditions. All estimations were made upon whole blood except the CO_2, which was made on the plasma.

A discussion of the various conditions will be taken up in the order in which they are presented in Table I. It is difficult to draw an arbitrary line indicating where normal findings end and pathologic begin, but it is believed safe, when the blood is taken after a 14-hour fast (in the morning before breakfast), to regard a urea nitrogen above 20 mg. and a sugar above 0.15 per cent as quite definitely pathologic, etc.

Renal Diabetes

It will at once be noted in the case of renal diabetes that the blood sugar is perfectly normal, and it is only when a knowledge of this fact is at hand that a definite diagnosis of this rather uncommon condition can be made. Here the threshold point of sugar excretion is below the level of the normal blood sugar. Broadly considered, renal diabetes may be regarded as a condition of glycosuria (glycuresis[4]) not dependent upon a temporary increase of blood sugar in an individual free from symptoms of diabetes mellitus. It should perhaps be noted that mild glycosuria occasionally appears to be associated with parenchymatous nephritis[5] in which case a slight hyperglycemia may be present, but quite without influence on the glycosuria.

Diabetes Mellitus

In diabetes the examination is primarily directed to the determination of the sugar, although in advanced cases the acidosis, as indicated by the CO_2, may assume greater significance. Here the condition of lipemia may develop, and of this the cholesterol is a particularly good index.

It should be remembered that the excretion of sugar by the kidney is simply one of the body's many factors of safety, in fact it may be compared with the safety valve of a steam boiler. The real condition to which attention should be directed is the hyperglycemia, and as the disease advances the glycosuria becomes less and less a safe criterion of this, since the permeability of the kidneys for sugar appears to be gradually lowered. Normally the blood sugar varies from 0.09 to 0.12 per cent and in early cases of diabetes one may note an excretion of sugar when the sugar of the blood rises above 0.16 or 0.17 per cent, but in advanced cases showing nephritic symptoms, blood sugar figures of 0.2 to 0.3 per cent, and even more, may be noted without the appearance of any sugar in the urine. While it is possible that the rise in the blood sugar threshold may not be chiefly dependent upon the development of renal disease, with cases of diabetes of long standing there is quite generally a rise in the sugar threshold. For this reason the urine sugar may frequently give misleading information in these cases. In those cases which develop a severe acidosis, the CO_2 determination of Van Slyke is a much more reliable and valuable index of the acidosis than any urinary determination. Except in the terminal stages with coma the acidosis appears to be almost entirely compensated, probably chiefly by the bicarbonate. This fact increases the clinical value of the CO_2-combining power. Any extended medical treatment or surgical interference should not be attempted on a severe diabetic without a knowledge of the blood sugar and the alkali reserve of the body as indicated by the CO_2 of the blood.

Although the fundamental function of the pancreatic hormone, insulin,

appears to be the regulation of carbohydrate metabolism, still it indirectly has an extremely important influence upon fat metabolism, and acidosis. Normally the oxidation of fat occurs along with that of carbohydrate. Without the oxidation of a certain amount of carbohydrate, there is an incomplete oxidation of fat, giving rise to the ketone bodies. This in turn produces an acidosis. As pointed out by Banting, Campbell and Fletcher,[6] insulin appears to be almost a specific for diabetic coma.

Gout

The blood in gout is characterized by its rather high content of uric acid, figures of 4 to 10 mg. It should be borne in mind, however, that early cases of interstitial nephritis disclose similar figures, although here there is generally a tendency for urea retention as well. Since a purine-free diet lowers the blood uric acid, treated gout cases may occasionally be encountered where the uric acid is only slightly elevated. However, in most cases of gout, the blood uric acid is not readily reduced by dietary restrictions in the purines. The estimation of the blood uric acid is a valuable aid to the diagnosis of gout, but only when considered in connection with the clinical symptoms and other laboratory findings.

Nephritis

There would appear to be little doubt that early cases of chronic nephritis are accompanied by an appreciable rise in the blood uric acid, although a rise in the blood urea can probably be taken as a safer sign of impaired kidney function. It is certainly true that the urea nitrogen falls within very narrow limits for perfectly normal individuals. As soon as one passes to hospital patients, however, figures above 15 mg. of urea nitrogen are found. Figures over 20 on the usual restricted diet of the hospital would suggest impaired kidney function. Creatinine appears to be more readily eliminated than either uric acid or urea, and it is not, as a rule, until the blood urea has doubled, or more than doubled the normal, that there is a very appreciable increase in this purely endogenous waste product derived apparently from muscle metabolism. The normal for the creatinine of the blood is approximately 1 to 2 mg. per 100 c.c. and figures over 3.5 mg. can be viewed with grave concern, while figures over 5 mg. are almost invariably indicative of an early fatal termination.[7] The only possible exceptions are cases where the retention is due to some acute renal condition, such as acute nephritis, intestinal obstruction, and mild bichloride poisoning. How these changes gradually come about is well brought out in the accompanying staircase table (Table II).

The inability to properly excrete the waste products of nitrogenous metabolism is only one of the difficulties which arise as the result of renal disease. As is well known, in parenchymatous nephritis, or nephrosis, the edema is probably dependent, in part at least, on the lowered permeability of the kidney for chlorides with their consequent retention. It is natural, therefore, to expect that the excretion of other salts should be deficient, although we have only recently come to appreciate the effect of the retention of phosphates. Normally, acid phos-

Table II

Staircase Retention of Uric Acid, Urea and Creatinine in Nephritis*

Date 1915-16	Case	Age	Sex	Diagnosis	Condition	Mg. per 100 c.c. of blood			Phthalein 2 hrs. per cent.	Systolic blood pressure	Urine	
						Uric acid	Urea N	Creatinine			Albumin	Casts
I												
9/17	H. L.	23	♂	Pulmonary tuberculosis	Unchanged	6.5	16	2.7	58	130	++	+
8/10	E. H.	41	♂	Pericarditis	Unchanged	5.6	13	2.1	45	150	–	–
10/12	F. D.	45	♂	Interstitial nephritis	Unchanged	5.5	12	2.5	37	185	–	+
3/ 6	B. D.	35	♀	Diffuse nephritis	Unchanged	9.6	19	2.4	45	175	+	+
II												
8/11	J. J.	65	♂	Early interstitial nephritis	Unchanged	9.5	25	2.5	13	185	+	+
7/21	D. S.	56	♂	Early interstitial nephritis	Unchanged	6.6	24	3.3	26	185	–	+
9/21	D. D.	52	♂	Early interstitial nephritis	Unchanged	8.7	20	3.6	20	100	+	+
8/ 3	C. M.	54	♂	Early interstitial nephritis	Unchanged	6.3	31	2.0	23	150	–	–
III												
1/ 6	L. P.	57	♂	Moderately severe chronic interstitial nephritis	Improved	8.0	80	4.8	0	240	++	++
3/ 1						4.9	17	2.9	10	170		
4/23	J. P.	34	♂	Moderately severe chronic diffuse nephritis	Improved	8.3	72	3.2	25	238	+++	++
5/21						5.3	21	1.9	43	145		
1/15	W. C.	49	♂	Moderately severe chronic diffuse nephritis	Improved	9.5	44	3.5	38	210	++	++
1/28						2.5	19	1.9	52	120		
IV												
4/11	E. C.	50	♀	Typical fatal case of chronic interstitial nephritis	Died	22.4	236	16.7	0	210	++	Pus
3/23	T. D.	34	♂	Typical fatal case of chronic interstitial nephritis	Died	15.0	240	20.5	2 - 3	225	++	+
1/25	S. H.	37	♂	Typical fatal case of chronic interstitial nephritis	Died	14.3	263	22.2	0	220	++	+
4/15	J. W.	34	♂	Typical fatal case of chronic interstitial nephritis	Died	8.7	144	11.0	Trace	225	+	+

*From Chace and Myers,[9] 1916.

phate provides one of the most important mechanisms of eliminating acid. When the phosphate excretion is impaired, bringing about an increase in the (acid) phosphate of the blood[8] (and tissues), an acidosis results, which may be quite as severe as that resulting from diabetic ketosis, judging from the CO_2-combining power of the blood. All cases of advanced interstitial nephritis suffer from acidosis,[10] and in some cases this is apparently the actual cause of death. It may also be noted that cases of acute nephritis occasionally show marked acidosis. When this is relieved by the administration of alkali or otherwise, a simultaneous clinical improvement takes place.

In parenchymatous nephritis, if we may be allowed to use this term, the findings are quite different. Here the nitrogen retention is comparatively small, although, as noted above, the examination of the blood may disclose a retention of chlorides. The figures for urea nitrogen seldom exceed 30 mg. except in terminal stages of the disease, and generally fall between that figure and 15.

These facts regarding the nitrogen retention and acidosis of nephritis are worthy of consideration from a surgical as well as from a medical point of view, especially when one considers that a very appreciable fall in the CO_2-combining power of the blood may result from the use of a general anesthetic such as ether. When the acidosis is already pronounced, it is easy to imagine what may happen from the additional fall of 10 to 15 volumes per cent, such a fall being not uncommon in individuals without acidosis as the result of ether anesthesia.

Urologic Conditions

Preoperative information on the chemistry of the blood should be secured in all surgical conditions of the kidneys, bladder or prostate.[11, 12] The information is of more value in prostatic than in other urologic lesions. Of the different chemical blood tests, the urea is the most helpful, although with urea retention, the creatinine or CO_2-combining power may be more significant.

Uncomplicated cases of prostatic obstruction show on the average urea nitrogen figures which are definitely above the normal of from 12 to 15 mg. per 100 c.c. of blood. The average is probably about 18 mg., with a range of from 11 to 25 mg. Cases showing urea nitrogen figures under 20 mg. per 100 c.c. of blood may be regarded as good operative risks as far as the kidneys are concerned. When the urea nitrogen figures are found between 25 and 50, the patient should be operated on with considerable caution, and best after a period of preliminary treatment directed to relieve the nitrogen retention. The blood urea and creatinine are taken as the index of the preoperative care. Patients with marked nitrogen retention who do not yield to treatment are suffering from advanced renal disease and are obviously poor surgical risks. With cases showing some impairment in renal function, a knowledge of the CO_2-combining power is of considerable preoperative value, since patients in whom there is a reduction in the alkaline reserve withstand operative interference better after receiving alkali medication.

The blood findings in vesical neoplasms, whether malignant or nonmalignant, are similar to those found in prostatic obstruction, the urea nitrogen

on the average being only slightly increased, if we except those cases which show definite evidence of nitrogen retention. Urea nitrogen figures of over 30 mg. appear to afford an unfavorable prognosis in carcinoma of the bladder, irrespective of the therapeutic procedure.

It is of interest to note that at the time when patients having a double polycystic kidney apply for medical aid, they almost invariably show quite marked nitrogen retention, the figures being comparable with advanced interstitial nephritis, although the retention of creatinine in proportion to the urea is higher, if anything, than in chronic nephritis.

While blood analyses indicate the condition of both kidneys only, they are nevertheless of considerable value in ascertaining the operability of patients with unilateral renal lesions. In such cases, the blood findings are usually within normal limits. However, in lesions necessitating nephrectomy, the toxemia produced by the renal infection sometimes temporarily impairs the function of the other kidney, so that the urea nitrogen may be 30 mg. or more. After the diseased kidney has been removed, the urea drops to normal. In bilateral nephrolithiasis in which nephrotomy and nephrectomy are indicated, the information obtained from the blood, coupled with the separate phthalein excretion of the two kidneys, is of great prognostic value.

Toxemias of Pregnancy

Recent data on the chemistry of the blood in the toxemias of pregnancy, [13, 14, 15, 31] eclampsia and hyperemesis, have considerably modified the older views regarding these toxemias, chiefly because they have given us definite information regarding renal function. In normal pregnancy the values for the nonprotein and urea nitrogen are slightly low and there is some decrease in the CO_2-combining power during the last months, but otherwise the blood is essentially normal. With true eclampsia, also hyperemesis, there appears to be only slight impairment in renal function, contrary to older assumptions. The nonprotein nitrogen is moderately increased, but the urea nitrogen is at the low normal limit or decreased, constituting 15 to 40 per cent of the nonprotein nitrogen instead of the normal 50 per cent. As a rule the uric acid is definitely increased, due apparently to a slight impairment in renal function. Taking the CO_2-combining power as the index there is a moderate or severe acidosis in all cases. Some cases show high blood chlorides and marked edema, others a slight but definite hyperglycemia. Judged both from the chemical changes in the blood and the clinical symptoms prompt improvement follows evacuation of the uterus in most instances. Toxemias do occur in which nitrogen retention exists but it seems probable that in most cases this is due to a true nephritis, which may have antedated the pregnancy. A high creatinine in such cases furnishes an unfavorable prognosis.

Pneumonia

The cyanosis of advanced pneumonia has long been recognized clinically and utilized as a prognostic sign. Recently the arterial and venous unsatura-

tion of the blood of pneumonia cases has been studied and correlated with the cyanosis.[16] Obviously the cyanosis is due to the incomplete saturation of venous blood with oxygen in the lung. The oxygen unsaturation of arterial blood normally averages 5 per cent and venous blood 25 per cent. With increasing cyanosis the arterial unsaturation becomes greater. The arterial unsaturation of fatal cases is always increased and may be very high (70 per cent). In such cases the venous unsaturation is extremely high (over 85 per cent).

It is well known that the development of severe pneumonia in human subjects often entails a more or less pronounced impairment of renal function, apparently secondary to the pneumonia. At the time of the crisis there is a definite increase in the nonprotein nitrogen of the blood,[17] due chiefly to a rise in the undetermined fraction. Following this the uric acid concentration of the blood generally rises and may reach 10 mg. With this there is usually an increase in the urea nitrogen to 20 mg. and more. When the urea nitrogen exceeds 20 mg. there is generally some creatinine retention. Occasionally the creatinine may exceed 5 mg. and death in these cases can be attributed in part at least to the severe impairment in renal function. Most pneumonia patients show a decrease in the blood chlorides before the crisis. Pneumonia likewise produces a slight decrease in the CO_2-combining power, 45 to 50 volumes per cent, although in severe cases with marked nitrogen retention it may be very low.

Malignancy

If cases of malignancy without renal complications are considered, the chemical blood findings are essentially normal,[18] or even subnormal, in the case of the nonprotein and urea nitrogen. This statement seems to apply more to superficial lesions than to cancer of the internal organs. With abdominal carcinomatosis, and carcinomata of the bladder, prostate, uterus, rectum and stomach the tendency to a secondary nephritis is pronounced,[19] A large percentage of these cases show a more or less severe impairment of renal function. The appearance and progress of this renal insufficiency follows the order of retention of the nitrogenous waste products characteristic of nephritis of the interstitial type.

It should perhaps be noted that in the early stages of malignancy there appears to be some tendency to a hypercholesterolemia, while several workers have observed a slightly increased alkalinity (increased pH) of the blood in malignancy, although a logical explanation of this latter fact is not apparent.

Infantile Conditions

In infantile tetany, rickets and the diarrheal acidoses, chemical blood findings have furnished data of special interest and significance. It has been recognized for some time that the tetany following parathyroidectomy in

dogs is associated with decreased blood calcium, and that the symptoms of tetany are relieved by calcium therapy. The calcium content of human blood serum is normally very constant at somewhere between 9 and 11 mg. per 100 c.c., the figures averaging slightly higher in children than adults. In infantile tetany for some unknown reason the calcium falls, figures between 3.5 and 7.0 mg. being found where symptoms of tetany are present.[20] Symptoms occur in outbursts as long as the calcium remains low. The tetany is relieved only by the persistent and constant administration of calcium, but with serum calcium figures of 7.5 mg. or more symptoms do not appear. There would appear to be some definite relation between the increased irritability of the neuromuscular mechanism, which is the essential phenomenon in infantile tetany, and the reduction in the calcium of the blood.

In normal infants and children the inorganic phosphorus of the blood averages slightly above 5.0 mg. per 100 c.c., although in adults the average is somewhat lower, about 3.7 mg. In active rickets the inorganic phosphorus of the serum appears to be regularly reduced (below 3.7 mg.) sometimes to an extreme degree[21] (2.0 mg.). A low phosphorus may be found without much sign of rickets, but rickets seems to be the most important clinical disorder associated with low phosphorus, and although the test cannot be considered pathognomonic of rickets, it is of distinct value in early diagnosis. Rickets appears to be occasionally encountered where the inorganic phosphorus is normal, but here the calcium is reduced. In experimental rickets in rats the phosphorus of the diet may be made the limiting factor in the production of rickets. Cod liver oil and ultra violet light, the two therapeutic agents most widely used in the treatment of rickets, both raise the blood phosphorus.

The acidosis occurring with infantile diarrhea has been the subject of a number of recent studies (see Chapter VI). Apparently the acidosis found in many cases of severe diarrhea not of the ileocolitis type is not due to the presence of acetone bodies, but rather to deficient excretion of acid phosphate by the kidneys, as in the case in nephritis. The administration of sodium bicarbonate will generally bring about a cessation of the almost characteristic hyperpnea and restore the CO_2–combining power to normal. Nevertheless in some cases the child may die. In cases of diarrhea without ileocolitis there is only a moderate increase in acetone bodies, while with ileocolitis the amount of acetone bodies is very large. In either condition the CO_2–combining power has been very helpful in pointing the way to an accurate diagnosis and therapy.

Fractures

As pointed out in connection with rickets, the inorganic phosphorus content of the blood of infants and children (during the period of bone growth) appears to average above 5.0 mg., while in well nourished adults it is somewhat lower, about 3.7 mg. It has recently been observed[22, 23] that following major fractures in adults there is a rise in the inorganic phosphorus content of the blood, in many instances to the level found in children.

There appears to be a similar slight change in the calcium. This rise may take from several days to two to four weeks. After union the phosphorus gradually falls. As a rule cases with non-union do not show this reaction in the blood phosphorus following facture.

Cholelithiasis

Since gallstones are largely composed of cholesterol, it is reasonable to suppose that their appearance might be associated with an increase in the cholesterol content of the blood. Although it seems quite probable that a hypercholesterolemia is present during the early period of the formation of the calculi, analytical data show wide variations in the blood cholesterol, [24, 25] the findings ranging from low normals to figures that are definitely increased. This being the case, it is not possible to employ the cholesterol estimation in the blood as a satisfactory means of diagnosing cholelithiasis.

A mild hyperglycemia, 0.15 to 0.25 per cent is quite frequently found in cases of cholelithiasis, especially when cholecystitis is present.

Pernicious Anemia

It is of interest that in pernicious anemia the cholesterol content of the blood plasma is markedly decreased.[24] When the antihemolytic action of cholesterol is recalled, it will be seen that this observation may possess some practical significance. The therapeutic administration of cholesterol in this condition has received attention from Italian investigators and has apparently been followed by beneficial results.

Miscellaneous Conditions

Sufficient has been said to indicate the practical value of the chemical analysis of blood in pathologic conditions, especially those of a constitutional nature, although many others might be mentioned. Disturbances of an endocrine origin have long been recognized to be associated with a change in carbohydrate tolerance. The estimation of the blood sugar after the administration of a suitable amount of carbohydrate has afforded a much more reliable method of bringing out deviations in tolerance than the older technic of examining the urine. In leucemia it is of interest that we frequently find a very marked increase in the uric acid content of the blood.

Differential Diagnosis

The physician encounters many conditions where a knowledge of the chemical blood findings is of great assistance in differential diagnosis. One of these has already been mentioned, viz., renal diabetes. Without a knowledge of the blood sugar content it is scarcely possible to suitably differentiate this condition from diabetes. The estimation of the blood uric acid is often of considerable assistance in the differential diagnosis of gout and arthritis, the uric acid being essentially normal in the latter condition. Cases are sometimes encountered which clinically might be diagnosed as cases of essential hypertonia without a

knowledge of the chemical blood findings, but these disclose a high uric acid, definite urea retention and figures for creatinine somewhat higher than one would ordinarily expect from the urea, a picture which appears fairly characteristic of nephritis of the arteriosclerotic type. In essential hypertonia one may find a fairly marked increase in the uric acid, but obviously no urea or creatinine retention. Cases of apoplexy present some of the clinical symptoms of uremia, but negative data on the urea and creatinine of the blood definitely exclude "uremia" as the cause of these symptoms. Such cases should not be passed over, however, without a knowledge of the CO_2-combining power, as cases of acute nephritis are sometimes encountered without nitrogen retention but with pronounced acidosis.[10]

Chemical Analysis of Blood

In the decade passed since the first introduction of delicate and relatively simple methods of blood analysis, a great variety of analytical procedures have been introduced, many of which yield very satisfactory results. Some of these methods, however, although simple, are quite technical and consequently give reliable figures only in the hands of those specially trained. Such methods, for example, as the hydrogen-ion concentration, calcium, etc., require more technical skill than others, but almost all the methods have individual pitfalls. While many of these estimations may be carried out by technical assistants under the direction of competent biochemists, individuals without suitable training in analytical and physical chemistry cannot independently be expected to secure entirely trustworthy figures. The chemical judgment which comes with such training is indispensable for those who find it necessary to do this type of biochemical work. Obviously a physician cannot expect that his office girl or nurse can properly manipulate these methods without an appreciation of the chemical factors involved.

With quantitative work, such as is required in the chemical analysis of blood, accurately prepared standard solutions and reagents are obviously the first essential. Although many of these are relatively stable, others keep for only a limited period. Knowledge of the preparation of such solutions and reagents and their reliability is quite as necessary as a knowledge of the methods themselves. One should also bear in mind that many glass instruments, such as pipettes, burettes, etc., unless specially purchased, require recalibration.

Despite the cautions that have just been raised, the author has had constantly in mind the limitations under which much of our clinical work must be done. In selecting the methods which are given at the end of the individual chapters, an attempt has been made to describe procedures which are simple, do not require expensive apparatus, furnish reasonably accurate information, and in which the chance for error is small.

In hospital work chemical blood analyses are now made so frequently and in such large numbers that it is important that the various procedures should be as simple as possible. As a step in this direction Folin and Wu have recently devised a system of chemical blood analysis (See Chapter

IX) in which a large number of analyses are made on the same protein-free filtrate. With this scheme, it is possible to make quite quickly a single or several fairly complete blood analyses. In a hospital laboratory, however, one is frequently called upon to make individual determinations of such substances as urea, sugar or CO_2-combining power on a very large number of specimens of blood. It is believed that the Folin-Wu system is a little less flexible than the individual methods we are about to describe (given at the end of Chapters II-VIII) although the different methods of the system may very well be employed independently. It should be borne in mind that blood chemistry is probably still in its infancy and improvements in technique are almost a daily occurrence.

Time of Taking Blood

It is quite essential that specimens be secured under as uniform conditions as possible. Fairly constant conditions are obtained by taking the blood in the morning before breakfast, i, e., after a 12 to 14 hour fast and before any food or fluid has been taken. If dietary treatment is to be instituted (as in cases of gout, nephritis, and diabetes) to be especially helpful the first specimens of blood must precede these dietary restrictions in order that they may give a reliable indication of the influence of the treatment.

Sometimes additional information may be obtained by taking a specimen a couple hours after the heavy meal of the day, but if only one specimen is to be secured, it is best to obtain this in a fasting condition. There is also the added advantage that the taking of blood specimens early in the morning permits the analysis the same day, which is quite necessary in the case of certain determinations.

Preservation of Blood

The question sometimes arises, how long can a specimen of blood be kept and have the analysis valid. If possible, specimens should always be analyzed the same day they are taken. In the case of the hydrogen-ion concentration the estimation should be made immediately after the withdrawal of the blood, the CO_2 content should be determined with little delay, while sugar and creatinine can best be estimated within an hour or two after the blood is drawn, especially with normal (low) findings. With these exceptions specimens can frequently be kept for several days without serious deterioration where good refrigeration is available. This statement does not apply, however, to specimens sent by mail which are at room temperature for several days. When blood is taken in a sterile container, it may be allowable for such specimens to be sent by mail if analysis can be made within 24 hours. Personal experience has shown, however, that such bloods generally show low blood sugars, a very good indication that deterioration has already set in.

Where sugar alone is to be determined, Denis and Aldrich[26] have found that one drop of "formalin" to 5 c.c. of blood acts as a satisfactory preserv-

ative (they used the Folin-Wu method). Such blood may also be used for creatinine and uric acid estimations, but the results obtained for nonprotein nitrogen and urea (urease method) are invariably too low. The Folin-Wu tungstic acid filtrate can be preserved for some little time with the aid of toluene, but this necessitates beginning the chemical analysis. Likewise with (the modified) Benedict's picric acid method for blood sugar described on page 92 the estimation of the sugar in the picric acid filtrate may be deferred to any convenient time. Several years ago the author had special small vacuum bottles constructed with the idea that these might be used for shipping blood, but they were found not to keep the blood cold for a sufficient length of time to be of material help.

Analysis of Whole Blood, Plasma or Serum

Although it is much more convenient, and desirable from the standpoint of uniformity, to carry out all the various blood analyses on whole blood, there are some practical as well as theoretical advantages for making some of the estimations on the plasma or serum. It may be said, however, that for ordinary clinical purposes whole blood is quite adequate for the estimation of the various nonprotein nitrogenous constituents, and sugar, chlorides, and cholesterol. This saves some time, is more convenient, and is considerably more economical from the standpoint of the quantity of blood used.

Berglund[27] has recently presented some interesting data giving figures for the various nonprotein nitrogenous constituents in whole blood, plasma and corpuscles. For the urea it makes comparatively little difference whether whole blood or plasma is used, although the plasma figures are slightly higher. In the case of the amino acid nitrogen, undetermined residual nitrogen, and the total nonprotein nitrogen, the figures for the whole blood are somewhat higher than for the plasma, and the corpuscles show higher values than either whole blood or plasma. Uric acid on the other hand appears to be chiefly a constituent of the plasma. For this reason the plasma uric acid may be much higher than the whole blood uric acid in cases with marked nitrogen retention (see page 147). In the case of creatinine there is only a relatively small difference in favor of the plasma, since the cells appear to be fairly permeable to creatinine.

Blood sugar resembles the creatinine in that there is comparatively little difference between the plasma and whole blood. In the case of the cholesterol, the variations are chiefly in the plasma (see page 124). Likewise with the chlorides the major portion is in the plasma and variations appear to occur chiefly in the plasma. Since there is some interchange of ions between the cells and plasma on standing, plasma chloride estimations should only be made on plasma that has been separated relatively soon after the blood is drawn (see page 133). The figures for plasma chloride are rather more significant than those on whole blood. However, if the plasma cannot be uniformly separated a short time after blood specimens are withdrawn, it is better to analyze whole blood, as pathological variations are readily brought out by such analyses.

In the case of such mineral determinations as calcium, plasma or serum should be used, and serum seems preferable. (Calcium is not present in the cells in significant amounts.) Oxalated plasma obviously cannot be employed owing to the precipitation of the calcium by oxalates. If plasma is to be used, the blood must be citrated. With the methods most commonly used at present, the calcium oxalate is precipitated directly, without ashing or removal of the protein, and consequently serum is to be preferred to plasma, as there is less danger of hemolysis in the separation of serum, and further the necessity of adding a large amount of salt (sodium citrate) as an anticoagulant is obviated.

Where inorganic phosphorus is to be estimated on the same blood as the calcium, serum is probably to be preferred to plasma.

For magnesium, sodium and potassium, serum is likewise preferable to plasma. In the case of potassium it should be borne in mind that, in contrast to sodium, this element is chiefly a cellular constituent. The amount in serum appears to be very constant, but the content in whole blood is about 10 times that of the serum, and varies roughly with the red cell count.

In blood gas analysis determination of carbon dioxide and oxygen) it is necessary to take rigorous precautions to prevent loss or absorption of these gases, when it is desired to estimate their actual content in arterial or venous blood. For such purposes the blood is generally drawn under mineral oil, potassium oxalate being used to prevent clotting. Where only the CO_2-combining power or the oxygen capacity are desired, less rigorous precautions need be used, but the blood should be analyzed as soon as possible. Although hemoglobin appears to be the chief carrier of carbon dioxide as well as oxygen, still the plasma is in general equilibrium with the cells, and it has been customary to determine the alkaline reserve (Van Slyke) on the plasma, chiefly for the reason that plasma gums up the Van Slyke apparatus less than whole blood. The CO_2-combining power determination is carried out after saturating the plasma with carbon dioxide at alveolar tension (alveolar air).

When the hydrogen-ion concentration of the blood is to be estimated, similar rigorous precautions must be taken to prevent the loss of carbon dioxide. In the case of the colorimetric estimation the oxalated blood must be constantly kept under oil, whether the estimation is to be carried out on the diluted plasma or on the dialysate from the whole blood.

Anticoagulants

When whole blood or plasma is to be analyzed, it is necessary to employ some anticoagulant. For chemical work oxalates have come to be almost universally employed. The mechanism of their action is simple, since oxalates precipitate calcium and calcium is necessary for the clotting of blood. Potassium oxalate has generally been employed on account of its solubility. The solubility of the sodium salt is much less (about 3.5 per

cent) than that of the potassium salt (about 25 per cent), and obviously the ammonium salt cannot be used for any analyses involving the determination of nitrogen. Folin has recently suggested the use of lithium oxalate, solubility about 6 per cent (see page 144), but it seems doubtful if its use will completely replace that of the potassium salt. (The potassium salt appears to interfere slightly in the estimation of uric acid.)

As Folin has pointed out 20 mg. of potassium oxalate are adequate for 10 c.c. of blood. Thus 2 drops of a 20 per cent solution are sufficient for 15 c.c. of blood and 4 drops for 25-35 c.c. We have been in the custom of adding 2-4 drops (generally 3 drops) to large necked bottles of 35 or 60 c.c. capacity (see Fig. 1). The bottles with the oxalate are then dried in a hot air oven at a sufficiently high temperature to practically sterilize them. They are then stoppered and kept on hand in quantity. By adding the oxalate in the laboratory in this way uniformity of quantity is secured. Drying removes the slight error introduced by the water and puts the oxalate in a finely divided state.

As pointed out above, serum or plasma are more adequate for the determination of the mineral elements than whole blood. For many purposes serum is to be preferred to plasma, because there seems to be less danger of hemolysis and further the introduction of a salt not only precludes the determination of one or more of the mineral constituents, but also seems to stimulate a change of the ions between the corpuscles and plasma.

Obviously potassium oxalate cannot be used where either calcium or potassium is to be determined. In such cases sodium citrate is most commonly used (see page 175).

Method of Taking Blood Specimens

For most chemical analyses it is necessary to obtain blood by venipuncture, although methods for several of the constituents (e. g., sugar, uric acid, urea) are sufficiently delicate that determinations may quite well be made on the quantities of blood obtainable by puncturing the finger or ear lobe. It should be borne in mind, however, that here one is really dealing with arterial blood. Some recent observations of Foster[28] are quite pertinent in this connection. He has observed that whereas the sugar content of both venous and finger blood is the same in the morning before breakfast, the sugar content of finger blood is much higher than venous blood after the ingestion of glucose. Evidently the glycogen forming function of the muscle stands between the arterial (finger) and venous blood.

The outfit illustrated below (Fig. 1) serves excellently for taking blood by venipuncture. It is similar to a device recently described by Cummer.[29] The outfit consists of a bottle of 35 to 60 c.c. capacity fitted with a two-hole rubber stopper, through which two glass tubes pass. One of these is made of heavy walled glass tubing, is slightly bent and is ground to receive a needle. To the other is attached a short piece of heavy, small bored rubber tubing, to which slight suction may be applied, if this is needed. The outfit

differs from the one we originally described[30] in 1915 in that the needle is attached directly to a rather short rigid glass tube instead of to a flexible rubber tube. This makes the manipulation of the needle slightly easier.

As noted above, potassium oxalate is generally used as the anticoagulant. To blood bottles similar to the one illustrated are added 2 to 4 drops of a 20 per cent solution of potassium oxalate, after which the bottles are dried in a hot air oven at a temperature sufficiently high to practically sterilize them. This insures a uniform amount of oxalate in the bottle in a finely divided state. The blood can best be drawn directly into the bottle, and must be *immediately* mixed with the oxalate by a gentle rotary motion (not by shaking). In case it is desired to analyze the plasma instead of the whole blood, this may readily be obtained by centrifuging the specimen.

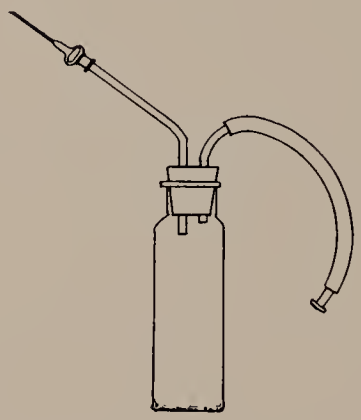

Fig. 1.—Outfit for taking blood.

Some prefer to draw the blood into a glass syringe, the barrel of which may be moistened with oxalate solution. If the blood can be immediately transferred to a tube or bottle with dry oxalate, moistening with oxalate solution is not essential. The use of a (small) syringe is especially advantageous where small amounts of blood are to be drawn repeatedly, as in the case of sugar tolerance tests.

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CHAPTER II

NONPROTEIN AND UREA NITROGEN

ALTHOUGH the nonprotein nitrogen normally constitutes only about one per cent of the total nitrogen of the blood, nevertheless greater interest is attached at the present time to variations in the bodies which form the nonprotein than the protein nitrogen. This is due largely to the fact that the variations in these nonprotein constituents give us an insight into some of the processes of anabolism and catabolism. The food nitrogen is carried by the blood to the various tissues and the waste nitrogen to the kidneys, directly or indirectly by the same medium. After a meal containing protein there is a temporary elevation in the nonprotein and amino nitrogen of the blood. In diseases of the kidney there may be at first only a slight rise in the uric acid or urea, although in the terminal stages of the disease there is generally a very marked elevation in all the forms of nonprotein nitrogen. The normal range of the various nonprotein nitrogenous components is given in Table I. Data are also included indicating the deviations which may occur in gout, interstitial and parenchymatous nephritis, and eclampsia.

TABLE I

NONPROTEIN NITROGENOUS CONSTITUENTS
Mg. to 100 c.c. of blood

CONSTITUENT	NORMAL	GOUT	EARLY INTERSTITIAL NEPHRITIS	TERMINAL INTERSTITIAL NEPHRITIS	PARENCHYMATOUS NEPHRITIS	ECLAMPSIA
				to		
Nonprotein N	25-35		30-50	350		35-55
Urea N	12-15		12-30	300	30-60	5-16
Uric acid	2-3	4-10	3-10	25		3-10
Creatinine	1-2		2-4	35		1-2.5
Creatine	3-7			30		
Amino acid N	6-8			30	12	4-8
Ammonia N	0.1			1		

The figures for the normal creatine are taken from observations of Denis,[1] those for amino acid nitrogen from Bock,[2] except in the case of eclampsia, where observations of Losee and Van Slyke[3] are recorded. Other data are from our own observations.

The figures for ammonia are very small, but these figures may be taken as the maximal rather than the minimal values. The very recent observations of Nash and Benedict[4] on the ammonia content of the blood (made on dogs and cats) give figures between 0.03 and 0.2 mg. to 100 c.c.

The data reported in Table II, taken from analyses made in Folin's laboratory by Berglund,[5] give the range in the nonprotein nitrogenous constituents of the whole blood, plasma and corpuscles of twelve normal men after a night's fast. From these figures it is evident that the nonprotein and urea nitrogen vary within comparatively narrow limits, when the blood is withdrawn in the morning before food has been taken. As urea is an extremely

diffusible substance, the slightly greater content in the plasma, would seem to find explanation in the smaller solid content. It will be noted that the content of amino acids and of the undetermined residual nitrogen, in particular, is much greater in the corpuscles than in the plasma. This difference in the undetermined nitrogen is too great to be explained on the basis of the known constituents, such as creatinine, creatine and uric acid. According to Berglund[5] the creatinine color reacting substance is normally fairly evenly distributed between plasma and corpuscles. The uric acid is likewise divided fairly evenly under normal conditions, although pathologically with high values it may be found chiefly in the plasma. Creatine is, however, present in the cells in approximately three times the concentration found in the plasma, but even this amount would account for only 1 to 2 mg. of the rest nitrogen. Concerning the possible nature of this residue, Folin and Berglund[6] have recently ventured the hypothesis that it consists, in part at least, of histons.

TABLE II

NORMAL MINIMUM, MAXIMUM AND AVERAGE BLOOD CONTENT OF NONPROTEIN NITROGENOUS PRODUCTS, ON TWELVE YOUNG MEN AFTER A NIGHT'S FAST*

		TOTAL NONPROTEIN N	UREA N	AMINO ACID N	UNDETERMINED RESIDUAL N
		mg. per 100 c.c.			
Whole Blood	Minimum	27.8	8.9	5.7	10.1
	Maximum	39.4	15.2	7.8	17.5
	Average	32.1	11.5	6.4	13.7
Plasma	Minimum	18.0	9.6	4.3	1.8
	Maximum	30.0	17.3	6.2	11.5
	Average	24.7	12.4	5.3	6.7
Corpuscles	Minimum	37.7	7.7	6.7	18.3
	Maximum	55.0	13.2	10.7	33.8
	Average	43.6	10.3	8.2	24.7

*Taken from Berglund[5].

Although the figures for the amino acid nitrogen are fairly constant after a night's fast, there is always a small increase after each protein meal. The top of the increase in the urea nitrogen after the meal comes five to seven hours later (probable deaminization period) and is accompanied by a rapid return of the amino acids to the starting level.

The origin and rôle which the various nonprotein nitrogenous constituents play in metabolism, as well as the ease of kidney secretion, obviously greatly influence the content of these substances in the blood, both normally and pathologically. Folin's classic papers on the composition of urine published in 1905,[7] did much to give us a correct appreciation of the significance of the nitrogenous waste products which find their exit through the kidney. He pointed out that the urea and creatinine stood in marked contrast to each other, since the former was largely exogenous in origin, while the latter was almost entirely of endogenous formation. Uric acid stood in somewhat of an intermediate position, being about half endogenous and half exogenous under ordinary conditions of diet.

Regarding the formation of these compounds, the following brief statement may be made. Urea is formed largely in the liver from the ammonia resulting

from the deaminization of amino acids set free in digestion, but not of immediate use to the animal organism. Uric acid originates as a result of the enzymatic transformation of the amino- and oxy-purines, in which various glands of the body participate. Creatinine would appear to be formed in the muscle tissue from creatine.

It is of interest to compare the partition of these nonprotein nitrogenous constituents in the blood with the similar partition in the urine. Upon the ordinary mixed diet their approximate distribution in the urine is 85 per cent urea N, 1.5 per cent uric acid N, 5 per cent creatinine N, 4 per cent ammonia N and

TABLE III

COMPARATIVE NITROGEN PARTITION OF URINE AND BLOOD
In per cent of total nonprotein nitrogen

FLUID	URIC ACID N	UREA N	CREATININE N	AMMONIA N	REST N
Normal Urine	1.5	85	5	4	4.5
Normal Blood	2	50	2	0.3	46
Blood in Gout and Early Nephritis	6	50	2	0.3	42
Blood in Parenchymatous Nephritis (Nephrosis)	2	55	2	0.3	40
Blood in Terminal Interstitial Nephritis	2 to 3	75	2.5	0.5	20

4.5 per cent undetermined N. It is quite natural to expect a somewhat similar relationship in the nonprotein nitrogenous constituents of the blood. The table above discloses quite a different distribution, however. It will be noted that even in normal blood the percentage of uric acid nitrogen is greater, if anything, than in the urine, while the urea is definitely lower, the contrast with the uric acid in the case of the creatinine and ammonia being even more marked. According to recent observations of Nash and Benedict[4] it seems likely that ammonia is not carried to the kidney for excretion by the blood, but formed in the kidney from urea in an effort to aid in the excretion of acid. The striking difference between the ability to excrete uric acid on the one hand, and urea and creatinine on the other, is brought out from an examination of the normal concentration of the blood and urine. Judging from their comparative composition, the kidney normally concentrates the creatinine 100 times, the urea 86 times, but the uric acid only 20 times. Myers, Fine and Lough[8] have pointed out that as the permeability of the kidney is lowered in conditions of renal insufficiency, this becomes evident in the blood, first by a retention of uric acid, later by that of urea, and lastly by that of creatinine, indicating that creatinine is the most readily eliminated of these three nitrogenous waste products, and uric acid the most difficultly eliminated with urea standing in an intermediate position.

Clinical Significance of the Blood Urea

Since urea is the chief component of the nonprotein nitrogen, and since its estimation is considerably simpler than that of the nonprotein nitrogen, our attention will be directed chiefly to the urea. Mosenthal and Hiller[9] have made

a careful study of the relation of the urea to the nonprotein nitrogen in disease. They point out that the selective action of the kidney maintains the urea nitrogen at a level of 50 per cent or less of the total nonprotein nitrogen of the blood, but that an impairment of renal function, even of very slight degree, may result in an increase of the percentage of urea nitrogen. In advanced cases this may be even higher than the 75 per cent given in Table III.

To give a comparative idea of the values observed for urea nitrogen in various pathologic conditions, illustrative findings are given for a number of different conditions in Table IV, the data being taken from actual cases. As will be noted, the conditions in which nitrogen retention may occur are quite

TABLE IV

CONDITIONS WITH SIGNIFICANT UREA NITROGEN FINDINGS

CASE	URIC ACID	UREA N	CREATININE	DIAGNOSIS
	mg. to 100 c.c.			
1	15.0	240	33.3	Bichloride poisoning
2	4.5	75	8.5	Double polycystic kidney
3	14.3	263	22.2	Terminal chronic interstitial nephritis
4	9.5	25	2.5	Early chronic interstitial nephritis, died 3 years later
5	8.3	72	3.2	Chronic diffuse nephritis, syphilis
6	2.3	28	1.9	Chronic parenchymatous nephritis
7	11.4	106	6.1	Severe acute nephritis, recovery
8	...	50	2.5	Mild acute nephritis
9	9.7	58	3.3	General carcinomatosis
10	5.5	24	3.3	Carcinoma of larynx
11	9.0	46	2.9	Severe pneumonia, recovery
12	..	43	3.4	Syphilis
13	5.5	44	3.1	Intestinal obstruction
14	..	24	2.2	Gastric ulcer
15	3.3	20	2.9	Duodenal ulcer
16	7.2	18	2.5	Prostatic obstruction
17	..	14	2.0	Myocarditis
18	6.0	18	2.9	Diabetes of long standing
19	8.4	12	2.2	Gout
20	6.8	7	2.2	Eclampsia

Strictly normal figures for urea nitrogen may be given as 12 to 15 mg. per 100 c.c. of blood, while figures above 20 mg. on the usual restricted diet of the hospital may be regarded as pathologic.[10]

numerous. Marked urea retention may occur not only in the terminal stages of chronic interstitial nephritis, but also in such conditions as bichloride poisoning and double polycystic kidney, and in some cases of acute nephritis. In parenchymatous nephritis the findings are comparatively low. Relatively high figures are frequently noted in malignancy, pneumonia, intestinal obstruction, lead poisoning, and sometimes in syphilis and cardiac conditions, although in the last mentioned this may be due to renal complications. In uncomplicated cases of prostatic obstruction the findings do not appear to be much above 20 mg. urea nitrogen. A slight retention is frequently noted in gastric and duodenal ulcer, possibly for the same reason that retention is found in intestinal obstruction. Advanced cases of diabetes frequently show definitely high figures, apparently due in some instances to the high protein diet, in others to a complicating nephritis. The fact that a normal urea is associated with a high uric acid is of practical value in cases of gout not complicated by nephritis.

Since urea is largely of exogenous origin, while creatinine is endogenous,

it is subject to much greater variation, especially under dietary influences. It is of less prognostic value than the creatinine in advanced cases of nephritis, but a much better guide as to the value of the treatment. In cases of prostatic obstruction the urea is an excellent pre-operative prognostic test, much better than the creatinine, for the reason that cases showing marked creatinine retention already show sufficient urea retention to make them very poor risks. Nephritis in children [11] does not so quickly result in urea retention as in the adult. On this account it is an especially helpful prognostic test in the nephritis occurring in early life.

Treatment of Nitrogen Retention

In conditions showing nitrogen retention there are obviously two lines of attack, (1) to increase the output of the kidneys and (2) to decrease the nitrogen intake, while still maintaining the caloric and other needs of the body. Until quite recently, the first method is the one that has been employed clinically, particularly with the use of such diuretic drugs as theobromine-sodium salicylate. For some time it has been recognized that in acute cases such drugs were contraindicated, and Christian [12] and his coworkers have further shown that these drugs are of very doubtful value in chronic cases. However, as Foster and Davis [13] have pointed out, some increase in the nitrogen output may be obtained by increasing the fluid intake. That a reduction in the nitrogen intake will reduce the blood retention has been definitely demonstrated, and this is a rational form of treatment which may be employed in almost all cases. In 1913 Goodall[14] reported observations on the favorable influence of a protein-free diet (Folin's starch and cream diet) in chronic nephritis. He found that such a diet could be continuously maintained for periods of from 5 to 10 days without harm to the patient, and further, that such a restriction could be followed by a low protein diet for a considerable period of time, even in advanced cases, without a return of the disagreeable symptoms. Later, Folin, Denis, and Seymour[15] conclusively proved that lowering the level of protein metabolism served to reduce the nonprotein and urea nitrogen of the blood in mild cases of chronic interstitial nephritis. Three years later the dietary end of this treatment was considered somewhat more in detail by Chace and Rose[16] in the wards and laboratory of this hospital. A number of different menus were outlined, the first two of which are given in Table V. Obviously neither of these should be continued for an extended period.

TABLE V

TWO DIETS FOR CASES OF SEVERE NEPHRITIS

Juice from 1 lemon, 2/3 cup water, 6 tablespoons lactose, 1 tablespoon cane sugar.	Served 4 times a day	
Calories, 1,424	Ash alkaline	Iron, 0.8 mg.

MORNING	NOON	EVENING
Banana ..... 300 gm.	Cream soup..... 200 c.c.	Banana ..... 300 gm
Cream ..... 100 c.c.	Banana ..... 300 gm.	Cream ..... 100 c.c.
Cocoa ..... 200 c.c.	Milk ..... 200 c.c.	Cocoa ..... 200 c.c.

Calories, 1,585 Protein, 35.4 gm. Ash, alkaline, 15.6 N Iron, negl.

To illustrate how a reduction in protein intake will lower the urea nitrogen, even in a very severe case of chronic interstitial nephritis, data on J. B. are

TABLE VI

CASE (J. B.) ILLUSTRATING THE INFLUENCE OF A LOW PROTEIN DIET ON UREA NITROGEN IN CHRONIC INTERSTITIAL NEPHRITIS

DATE 1917	URIC ACID	UREA N	CREATININE
	mg. to 100 c.c.	mg. to 100 c.c.	mg. to 100 c.c.
Jan. 5	....	135	9.7
Jan. 9	....	110	12.5
Jan. 16	....	93	9.5
Jan. 30	5.0	48	7.8
Feb. 16	5.4	56	9.1
Feb. 27	7.1	45	7.5
Mar. 6	6.1	49	6.4
Mar. 23	....	40	7.7
Apr. 13	5.9	46	6.4
Apr. 27	....	45	7.5
May 15	....	33	7.3
May 22	....	24	7.3
June 5	....	34	6.7
July 17	....	29	6.8

Patient left hospital clinically improved on June 9, returned to work as guard on the subway, but died Nov. 7, 1917.

given in Table VI above. Despite the fact that the (endogenous) creatinine could not be appreciably lowered, the urea nitrogen gradually fell to a level not more than twice the normal.

Index of Urea Excretion

As far back as 1903 Widal recognized the prognostic value of the blood urea, observing that patients with blood ureas of 4 to 5 gm. (per liter) survived only a limited period. In 1910 Ambard, undoubtedly influenced by these observations of Widal, attempted to estimate the functional ability of the kidneys in normal and pathological conditions by a study of the ratio between the urea of the blood and urine. This culminated in his attempt to express the rate of urea excretion in the form of mathematical formulæ. The work of Ambard [17] and his co-workers has attracted a great deal of interest not only in France, but also in this country. McLean, and Addis and Watanabe, in particular, have devoted considerable attention to this subject.

In general the arguments advanced by Ambard in favor of this method of studying kidney function are logical, but our own experience with the method has been disappointing in that the results have failed to reveal information not given by the blood alone.[18,19] Recently, Austin, Stillman and Van Slyke[20] have made an effort to derive a formula which would more correctly express the various factors involved. They have pointed out that the excretion rate in normal men increases directly as the blood urea, and as the square root of the volume of urine, so long as the latter remains within normal limits, under 5 liters per day. These relations are expressed in the formula, $D = KB\sqrt{V}$, D representing the rate of urea excretion calculated as gm. per 24 hrs., B the blood urea concentration (gm. per liter), V the rate of urine excretion,

calculated as liters per 24 hrs. With the size of the individual (W=wt. in kg.) incorporated in the formula it may be expressed, $K=\frac{D}{B\sqrt{VW}}$. For normal individuals $K=7.5\pm3$. With deficiency in the urea excreting function K has a lower value. According to Austin, Stillman and Van Slyke, the above formula gives more consistent results than that of Ambard, which assumes erroneously that the excretion rate increases as the square rather than the first power of the blood urea; and correlates the excretion with the concentration of the urea in the urine rather than with the volume of urine.

Obviously if this method of studying the urea excretion is used, it is essential that accurate collections of urine be made for the 60 or 72 minute specimen employed. This can be done if sufficient attention is given to the matter, but practically it seems difficult of execution in most hospitals. Austin, Stillman and Van Slyke make the practical suggestion that such errors, resulting from retention in the bladder or other factors may be reduced by basing the output calculations on the creatinine centent of the sample, rather than the time over which it is collected, since Shaffer has shown the hourly creatinine output to be constant throughout the 24 hrs., e. g., if the creatinine content of the sample analyzed is 1/20 of the individual's known daily creatinine output, the urea and volume output are calculated to a 24-hour basis by multiplying by 20.

As a result of their studies Austin, Stillman and Van Slyke conclude that the greater constancy of results calculated by their equation (given above) indicates the probability that it expresses the influence of the chief factors governing excretion with a closer degree of accuracy than does Ambard's equation. Their work affords a basis for detecting abnormalities in urea excretion, but not for interpreting the significance of such abnormalities, since it was largely physiological in its nature.

There are a number of conditions aside from nephritis where nitrogen retention is a factor of considerable clinical interest and importance. Reference has already been made to these conditions and findings in illustrative cases given in Table IV. Several of these are of sufficient importance to merit separate discussion, notably eclampsia, prostatic obstruction and intestinal obstruction.

Eclampsia

Chemical blood findings in normal pregnancy and eclampsia, taken from analyses of Killian,[21] are given in Table VII. As will be noted there is a tendency toward low values for both the nonprotein and urea nitrogen in normal pregnancy, while in eclampsia the values for the nonprotein nitrogen are either high normals or definitely increased, despite the fact that the urea nitrogen is either normal or subnormal. The urea nitrogen thus forms an abnormally low percentage of the total nonprotein nitrogen. As might be expected from this the undetermined nitrogen fraction is very high. Although the nature of this fraction has not been explained, it would seem that it might possess some clinical significance. It is of interest in this connection that

TABLE VII

CHEMICAL CHANGES IN THE BLOOD IN NORMAL PREGNANCY AND ECLAMPSIA

CASE	AGE	PARA.	GESTATION	BLOOD ANALYSES								REMARKS
				NON-PROTEIN N	UREA N	$\frac{\text{UREA N}}{\text{N-P N}}$	URIC ACID	CREATININE	SUGAR	CHLORIDES AS NaCl	CO_2 COMBINING POWER	
	yrs.		mos.	mg. to 100 c.c.		per cent	mg. to 100 c.c.		per cent			
1. N. C.	25	I	7½	25	11	45	2.6	2.1	0.14	0.46	42	
2. A. S.	23	I	8	24	11	47	2.6	2.5	0.10	0.48	49	Normal pregnancies.
3. E. Q.	27	I	8	22	10	44	2.5	2.4	0.11	0.48	44	
4. B. W.	25	I	9	30	5	16	8.5	...	0.10	0.63	16	Convulsions 3 hrs. postpartum.
5. H. S.	22	I	9	48	15	32	7.3	1.8	0.13	0.46	28	Convulsions 2 hrs. postpartum.
				26	13	50	3.5	2.1	0.12	0.50	40	14 days later, improved.
6. M. E.	27	I	7	34	13	36	11.0	2.5	0.13	....	38	Severe convulsions, stillbirth, died.
7. Y. H.	28	I	7½	35	9	27	6.8	...	0.12	....	38	Severe convulsions, stillbirth.
				23	12	51	2.5	...	0.09	....	53	5 days later, improved.
8. D. M.	22	I	8	37	11	29	5.0	...	0.14	0.47	37	Severe convulsions, fetus toxic.
9. F. H.	27	I	7	38	10	29	3.4	...	0.10	0.61	12	Severe convulsions, edema, died.
10. A. B.	21	I	9	50	9	18	5.6	2.2	0.18	0.52	21	Very severe convulsions, fetus toxic.

there appears to be a similar high rest nitrogen fraction in many cases of severe pneumonia. In eclampsia the creatinine is normal and Losee and Van Slyke[3] have also found this to be the case with the amino acid nitrogen. The uric acid is generally high, but this rarely accounts for more than 3 mg. of the nitrogen.

Considering the above findings it is apparent that true eclampsia shows only slight evidence of impaired renal function, indicated chiefly by the high uric acid. The normal or subnormal values for the urea are quite striking. Although toxemias do occur in pregnancy where the kidneys are involved, it seems probable that in many instances this renal factor antedates the pregnancy.

Prostatic Obstruction

Preoperative information on the chemistry of the blood has been found of great value in all surgical conditions of the kidneys, bladder or prostate, but it seems to be of special value in prostatic obstruction. Of the different chemical blood tests, the urea is the most helpful, although with urea retention, the creatinine or CO_2-combining power may be more significant. When the urea nitrogen is 20 mg. or under, the renal factor can be disregarded. Uncomplicated cases show on the average urea nitrogen figures which are definitely above the normal of 12 to 15 mg. per 100 c.c. of blood. The average is probably about 18 mg., with a range of 11 to 25 mg.

TABLE VIII

ILLUSTRATIVE CHEMICAL FINDINGS IN UNCOMPLICATED CASES OF PROSTATIC OBSTRUCTION

CASE	AGE	DATE	BLOOD ANALYSES			
			UREA NITROGEN	CREATININE	SUGAR	CHLORIDES AS SODIUM CHLORIDE
			mg.	mg.	per cent	per cent
1. M. K.	54	4/20/21	13.2	1.8	0.125	.....
2. C. D.	60	5/ 2/21	12.0	1.8	0.100	.....
3. A. R.	57	5/ 6/21	13.1	2.1	0.121	.....
4. J. C.	45	8/26/21	21.3	...	0.166	.....
5. L. V.	63	9/10/21	29.0	2.0	0.172	0.450
		9/12/21	24.9	2.6	0.142	
6. R. T.	66	9/15/21	13.9	...	0.166	0.475
7. S. L.	59	10/13/21	15.0	...	0.187	0.475
8. W. K.	49	10/17/21	19.5	...	0.150	0.563
9. L. M.	60	11/22/21	12.4	...	0.136	0.500
10. G. D.	67	11/23/21	22.5	3.3	0.150	0.500
		11/25/21	20.4	3.1	0.103	
11. H. F.	74	11/28/21	10.2	...	0.150	0.488
12. M. McG.	67	12/ 7/21	11.4	...	0.136	0.525

Illustrative chemical blood findings on twelve uncomplicated cases of prostatic obstruction, taken from data of Squier, Bandler and Myers[22] are given in Table VIII. In two of the cases the urea findings were sufficiently high to make it desirable to secure a second blood analysis after a couple of days of treatment. All of these cases were operated on satisfactorily without complications.

Patients presenting urea nitrogen figures over 25 mg. should be operated

on with caution, and best after a period of preliminary treatment directed to relieve the nitrogen retention. These cases are not the best operative risks, but a number of such cases have been operated on with satisfactory results after a more or less prolonged period of preoperative treatment, which favorably influenced the blood urea. Data on four such cases are given in Table IX. Patients with marked nitrogen retention who do not readily yield to

TABLE IX

THE INFLUENCE OF PREOPERATIVE TREATMENT ON THE BLOOD UREA IN PROSTATIC OBSTRUCTION; PROSTATECTOMY WITH GOOD RESULTS

CASE	AGE	DATE	BLOOD ANALYSES					SUPRAPUBIC PROSTATECTOMY
			UREA NITROGEN	CREATININE	SUGAR	CARBON DIOXIDE	CHLORIDES AS SODIUM CHLORIDE	
			mg.	mg.	per cent	c.c.	per cent	DATE
1. J. S.	60	4/22/20	46.6	4.3	.....	..	.....	5/ 5
		4/27/20	42.6	4.8				
		5/ 4/20	19.0	2.4				
2. S. M.	62	7/ 7/20	51.0	3.2	0.128	42	.....	7/12
		7/16/20	49.0	2.6	0.100	43		
		7/29/20	29.0	2.4	0.128			
3. J. McD.	61	5/11/21	28.8	3.5	0.114	..	0.507	5/19
		5/18/21	24.9	2.6	0.115			
		6/ 1/21	22.5	...	0.150	..	0.450	
4. G. B.	62	3/23/22	52.0	5.3	0.166	44	0.575	4/17
		3/25/22	55.9	8.9	0.170	..	0.600	
		3/29/22	35.0	5.0	0.140	47	0.563	
		4/ 1/22	37.5	7.5	0.133	48	0.553	
		4/ 7/22	20.6	5.0	0.130	..	0.500	
		4/12/22	23.4	4.5	0.138	62	0.538	
		4/15/22	22.0	4.2	0.109			

treatment are suffering from advanced renal disease and are obviously poor surgical risks. Operative interference almost invariably provokes a rise in the blood urea in uncomplicated cases of prostatic obstruction. It is easy to comprehend this effect on a badly damaged kidney.

Intestinal Obstruction

The increase in the nonprotein and urea nitrogen of the blood following intestinal obstruction in man was first pointed out by Tileston and Comfort[23]. Later Cooke, Rodenbaugh and Whipple[24] studied experimentally the chemical changes in dogs following intestinal obstruction. In addition to confirming the findings of Tileston and Comfort, these authors demonstrated that the intoxication observed in intestinal obstruction was in great part due to a proteose, which was comparatively easily isolated from the closed loop of intestine in animals by means of 95 per cent alcohol or ammonium sulfate, and was very toxic. This same proteose would appear to be present in the exudate from peritonitis in man. Rabinowitch[25] has shown that not only in intestinal obstruction, but also in acute general peritonitis, such as may arise from a perforated appendix, the blood urea is markedly elevated above the normal, in spite of comparatively normal kidney function. He regards the maintenance of a high blood urea nitrogen, in the presence of a good kidney function, as indicative of an unfavorable prognosis.

TABLE X

CHEMICAL BLOOD FINDINGS IN INTESTINAL OBSTRUCTION

CASE	AGE	DATE	BLOOD ANALYSES							LOCATION OF OBSTRUCTION, OUTCOME
			NONPROTEIN N	UREA N	CREATININE	URIC ACID	SUGAR	CHLORIDES AS NACL	CO_2 COMBINING POWER	
			mg. to 100 c.c.				PER CENT		C.C.	
1. B. O.	62	1/25/21		51	5.6		0.238	0.419		Large intestine, died.
2. V. H.	45	1/20/21		103	7.5		0.145	0.544	48	Strangulated hernia, died p. o.
3. I. H.	60	5/22/21		63	17.0		0.213	0.613	36	Small intestine, died.
4. A. P.	47	12/29/20		107	13.0		0.172	0.600	17	Small intestine, died.
5. N. W.	43	12/19/21	51	18	3.5	5.9	0.115		41	Strangulated hernia, preoperative.
		12/21/21	60	21	3.9	4.4	0.107		46	Postoperative, necrotic gut not resected.
		12/24/21	83	34	5.2	9.2			65	Alkali therapy, died.
6. M. D.	40	12/25/21	63	30	4.7	7.2			60	Small intestine.
		12/26/21	56	26	3.1	6.5			47	Postoperative.
		12/28/21	57	21	3.0	6.1			63	Improved.
7. S. P.	40	11/ 5/21		51	2.2	10.9	0.150			Small intestine, preoperative.
		11/ 6/21		50	2.1	12.2	0.160			Postoperative.
		11/ 8/21		32	2.1	9.1	0.136			
		11/13/21		15	2.0	3.8	0.136			
		11/20/21		12	2.0	4.0	0.125			Improved.

Unpublished observations of Killian.

The unpublished observations of Killian on cases of intestinal obstruction, given in Table X well illustrate the findings which may be obtained in severe cases.

Estimation of Nonprotein Nitrogen

The use of acetone-free methyl alcohol was first suggested by Folin and Denis[26] as the protein precipitant in the nonprotein nitrogen estimation. With this precipitant, the results, as Greenwald has pointed out, are somewhat low for the reason that amino acids appear to be partly precipitated. Another objection to the use of methyl alcohol is that some lipoid nitrogen is included in the filtrate. On this account Greenwald[27] suggested the use of trichloracetic acid. Subsequently Folin and Denis[28] recommended the use of *m*-phosphoric acid, although in their system of blood analysis Folin and Wu[29] now use tungstic acid. Folin[30] has stated that he prefers that precipitant which gives the lowest results without lowering the values of any known constituent.

The recent studies of Hiller and Van Slyke[31] tend to support Folin's claim regarding this reagent, i. e., that it does not carry down constituents ordinarily determined. Although tungstic acid brings down less amino acid nitrogen than trichloracetic acid, especially when the latter is employed in a 5 per cent concentration, still the tungstic acid precipitates the peptid nitrogen much more completely. When for any reason it is desired to determine the peptid nitrogen, trichloracetic acid would appear to be the preferable reagent.

Originally Greenwald recommended the use of a 2.5 per cent solution of trichloracetic acid, but later employed a 5 per cent solution. If one cares to include as much peptid nitrogen as possible, the original dilution is probably to be preferred. The figures for nonprotein nitrogen obtained with the latter, however, agree more closely with those obtained on the tungstic acid filtrate.

After the removal of the protein constituents it is necessary in any case to carry out a micro-Kjeldahl digestion on the nonprotein nitrogenous material. The ammonia may then be aerated or distilled off and finally estimated either colorimetrically with Nessler's solution or titrated with the aid of 0.01 N acid and alkali. In the recent method of Folin and Wu the digested material is nesslerized directly. Where distillation is employed the removal of the ammonia is somewhat quicker and probably slightly more complete, but the setting up of a suitable distillation apparatus is considerably more complicated. Nesslerization is a much more delicate procedure than acid-alkali titration, but when sufficient material can be employed, the latter is undoubtedly more accurate. However, if one works with 0.02 or 0.01 N acid and alkali solutions it is necessary to very frequently restandardize them, especially the alkali.

We have found the trichloracetic acid precipitation of Greenwald very satisfactory, although as a protein precipitant, it is mechanically hardly the equal of Folin and Wu's tungstic acid. After aeration we have carried out the final estimation colorimetrically as originally suggested by Folin and Denis.

Method.—Three c.c. of blood are diluted to ten times the volume (30 c.c.) with 5 per cent trichloracetic acid solution. (The 2.5 per cent solution may be obtained by first

diluting the blood to 15 c.c. with water and then making up to the 30 c.c. volume with 5 per cent trichloracetic acid.) After thorough mixing, it is allowed to stand for thirty minutes, then filtered. With the above treatment sufficient filtrate is generally obtained (20 c.c.) so that duplicate determinations may be made if desired. (In case one prefers the tungstic acid precipitation, this technic is given in connection with the determination of uric acid in the next chapter, also in Chapter IX.)

Into a thin glass test tube about 150 mm. in length and of diameter (20 mm.) such that it will readily slip into a 100 c.c. cylinder, are pipetted 10 c.c. of the filtrate, the equivalent of 1 c.c. of blood. Approximately 0.2 gm. of potassium sulfate, 2 drops of 10 per cent copper sulfate solution and 0.3 to 0.5 c.c. of conc. sulfuric acid, all of the highest purity reagents (nitrogen-free), are added and the mixture boiled over a small microburner flame, first gently to remove the water (this may be done very conveniently by placing the tube in a beaker of boiling saturated calcium chloride solution) and then until digestion is complete, i. e., two minutes after the mixture becomes colorless. The final oxidation may be greatly facilitated by the addition of 1 or 2 drops of hydrogen peroxide. The tube is allowed to cool for a couple of minutes and then about 6 c.c. of distilled water added.

Aeration of the ammonia may be conveniently carried out in the apparatus* (Fig. 2), described by the author in 1914.[32] This apparatus has two important advantages over that originally suggested by Folin and Denis for this purpose; first, it is not necessary to insert

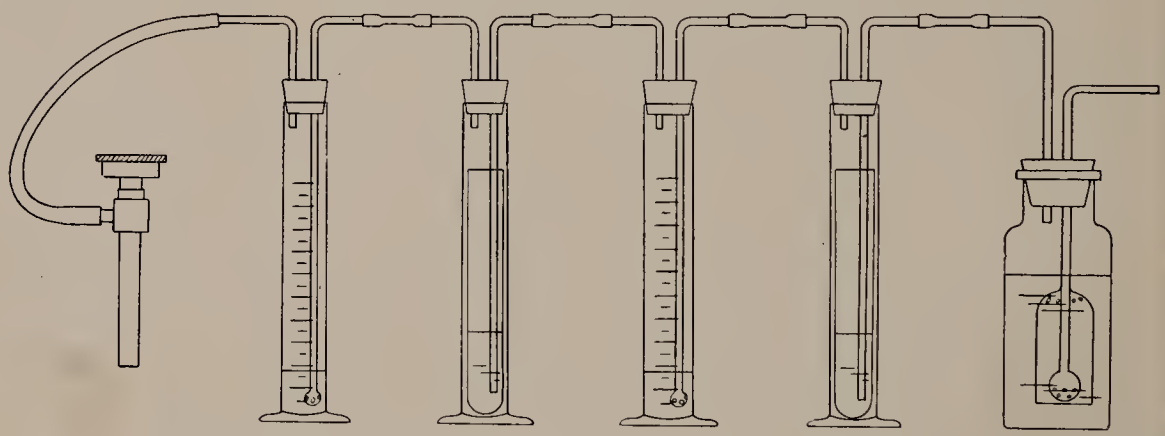

Fig. 2.—Aeration apparatus for nonprotein and urea nitrogen estimations.

a stopper into a fragile test tube, and second, nesslerization is carried out directly in the second cylinder, which allows a wide range of dilution in contrast to the fixed dilution of a volumetric flask. (The aeration of the ammonia from the urea, described below, is carried out with the same apparatus.)

Into a 100 c.c. graduated cylinder without lip are added 15 c.c. of distilled water and 2 c.c. of 0.1 N hydrochloric acid or 2 to 3 drops of the 10 per cent solution. This is now closed with a two-hole stopper having a glass tube passing nearly to the bottom of the cylinder. The tube is sealed at the lower end, but contains a number of small holes to aid in the complete absorption of the ammonia. The apparatus to connect the pair of cylinders is adjusted, the test tube containing the digested mixture placed in the ungraduated cylinder, 3 c.c. of saturated sodium hydroxide carefully allowed to run down the side of the tube and to the bottom of the acid solution and the stopper quickly inserted. The cylinder containing the digested mixture is connected with a wash bottle containing dilute sulfuric acid so that the incoming air will be completely ammonia-free. The outlet tube in the graduated cylinder is now connected with a suction pump and air slowly allowed to pass through the apparatus, the speed being increased so that at the end of two minutes

*This aeration apparatus may be very simply constructed provided 100 c.c. cylinders without lips are available. The holes in the ends of aeration tubes, as Folin has pointed out, may readily be made with a platinum wire which is at white heat, provided the glass is only moderately hot. Malleable iron wire works quite as well as the platinum. The cylinders or the apparatus complete may be obtained from the C. M. Sorensen Co., Inc., New York City.

the air current is as rapid as the apparatus will stand. A series of a half-dozen or more tubes may conveniently be set up in this way. Aeration is complete in 10 to 30 minutes and the apparatus is then disconnected.

Into a volumetric flask of 100 c.c. capacity are pipetted 5 c.c. of ammonium sulfate solution* containing 1 mg. of nitrogen and 50 to 60 c.c. of distilled water are added. Sufficient of the modified Nessler's solution** for the standard and the unknown is diluted about five times with distilled water, and of this about 20 c.c. added to the standard solution, which is then made up to the mark with water. At the same time 7 to 8 c.c. of freshly diluted Nessler's solution are added to the unknown and the volume made up to 25 c.c. in the graduate, unless a high content of nonprotein nitrogen is indicated, in which case more Nessler's solution (up to 25 c.c.) and a dilution to 35, 50, 100 c.c. or even more may be needed to make the color of the unknown approximately the same intensity as the standard. This is usually set at the 15 or 20 mm. mark on the colorimeter (Bock-Benedict, Duboscq or Klett). The colors are now matched up, preferably with the aid of a north light.

Calculation.—The following formula may be used for the calculation (applies to all three colorimeters), $\frac{S}{R} \times \frac{D}{100} \times 1 \times 100$=mg. nonprotein N to 100 c.c. of blood in which S represents the depth of standard, R the reading of the unknown, D the dilution of the unknown, 100 the dilution of the standard, 1 the strength of the standard in mg. and 100 the factor to convert the result to mg. per 100 c.c., since the equivalent of 1 c.c. of blood was employed.

For example with the standard set at 15, a reading of 16.2 and a dilution of 35 the formula would work out as follows:

$$\frac{15}{16.2} \times \frac{35}{100} \times 1 \times 100 = 32.4 \text{ mg. nonprotein N to 100 c.c. of blood.}$$

Estimation of Urea Nitrogen

The method for the estimation of urea nitrogen is one that we have constantly employed for the past ten years,[8, 32] and although other methods have been tried from time to time, none has been found as expeditious or as satisfactory. With a good preparation of urease the results obtained in the estimation of urea are perhaps the most reliable of any of the chemical blood determinations. The method is based on suggestions of Marshall,[33] of Van Slyke[34] and of Folin.[26]

The apparatus employed for the urea nitrogen estimation is the same as for the nonprotein nitrogen and if desired the two determinations may be run at the same time.

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*A standard solution containing 1 mg. of N per 5 c.c. of solution may be prepared by dissolving 0.944 gm. ammonium sulfate or 0.764 gm. ammonium chloride of the highest purity in distilled water and making up to 1,000 c.c.

**Modified Nessler's Solution (Bock and Benedict Formula).—Place 100 gm. mercuric iodide and 70 gm. potassium iodide in a liter volumetric flask and add about 400 c.c. of water. Rotate until solution is complete. Now dissolve 100 gm. sodium hydroxide in about 500 c.c. water, cool thoroughly, and add with constant shaking to the mixture in the flask; then make up with water to the liter mark. This usually becomes perfectly clear. When the small amount of dark brownish red precipitate, which forms, settles out, the supernatant fluid is ready to be poured off and used.

When pure mercuric iodide can not be obtained Folin and Wu have pointed out that metallic mercury and iodine may be substituted. To 75 gm. of potassium iodide and 55 gm. iodine in a 500 c.c. flask add 50 c.c. of water and an excess of metallic mercury, 75 gm. Shake the flask for 7 to 15 minutes or until the dissolved iodine has nearly disappeared. When the red iodine solution has begun to become visibly pale, though still red, cool in running water and continue the shaking until the reddish color of the iodine has been replaced by the greenish color of the double iodide. Now separate the solution from the surplus mercury by decantation and washing with about 400 c.c. of distilled water. Add 500 c.c. of water containing 100 gm. of sodium hydroxide as in the first formula and make up to 1 liter.

Method.—Into a test tube of the same size as that employed for the nonprotein nitrogen are introduced 1 c.c. of a 5 per cent jack bean urease solution* (and 2 drops of the activating phosphate solution if this is not already present). Two c.c. of the oxalated blood are now added, preferably with an Ostwald-Folin pipette and the tube (or series of tubes) incubated in a beaker of water at 50° C. for 15 minutes. At the end of this time the aeration apparatus is put in order, 1 to 2 c.c. of amyl alcohol (or 4 to 5 drops of pure caprylic alcohol) are added and then about 4 to 5 c.c. of saturated sodium carbonate. The tube is at once inserted in the ungraduated cylinder, the stopper replaced and aeration carried out for about 30 minutes, the air current being allowed to run slowly at first, but later as rapidly as the apparatus will stand.

The development of the color and comparison in the colorimeter is carried out just as described above, but in making the calculation it should be borne in mind that 2 c.c. of blood have been employed. If it is desired to convert the urea nitrogen into terms of urea, this may be done by multiplying by the factor 2.14.

Where only an occasional estimation of urea is carried out, the aeration procedure may appear a little troublesome. In an attempt to simplify this step we have tried a number of different protein precipitants, but *in no case have the results been quite as satisfactory as with aeration.* We originally suggested the use of heat and acetic acid together with colloidal iron, but later abandoned this in favor of *m*-phosphoric acid. Picric acid, and a weak acetic acid solution of potassium mercuric iodide were also tried, but recently Folin and Wu's tungstic acid has been employed. This reagent is the best protein precipitant that we have used in this connection.

The blood is digested in the usual way and then diluted 1 to 10 with water and the precipitation reagents. After filtration 5 c.c. of the diluted Nessler's solution are added to 5 c.c. of the filtrate (the equivalent of 0.5 c.c. of blood) and color comparison made in the usual way. Turbidity seldom develops when good sodium tungstate is used and the ⅔ normal sulfuric acid is of the correct strength. The results compare favorably with those obtained by the aeration method.

The technic of the method is described below in connection with the estimation of urea with the test tube colorimeter, given below.

Estimation of Urea with the Test Tube Colorimeter

Method.—Into a large test tube (160 × 25 mm.) are introduced 1 c.c. of 5 per cent urease solution or about 0.1 gm. of the dry enzyme. With an Ostwald-Folin pipette 2 c.c. of the oxalate blood (or 1 c.c. if a large amount of urea is anticipated) are now added and the tube incubated in a beaker of water at 50° C. for 10-15 minutes. (If desired the aeration described above may be used with the test tube colorimeter.) At the end of this time 13 c.c. (or 14 c.c.) of water are added and 2 c.c. of 10 per cent sodium tungstate, and then while rotating the tube, 2 c.c. of 2/3 N sulfuric acid. Shake vigorously. When a blood is properly coagulated, the color of the coagulum turns from pink to dark brown.

*Concentrated preparations of jack bean urease may be obtained on the market (Arlington, Squibb), prepared according to the Van Slyke formula, and have the activating phosphates already added. Folin has pointed out that the keeping power of these preparations is not constant, although we never experienced difficulty with them until quite recently. Folin suggests the following method of preparation: Transfer to a 200 c.c. flask about 3 gm. of permutit powder. Wash this by decantation, once with 2 per cent acetic acid, then twice with water. Add to the moist permutit in the flask 100 c.c. of 15 per cent alcohol (16 c.c. of 95 per cent alcohol mixed with 84 c.c. of water). Then introduce 5 gm. jack bean meal (may be obtained from the Arlington Chemical Co.) and shake gently but continuously for 10 to 15 minutes. Filter and collect the filtrate in three or four different small clean bottles. Set one aside for immediate use: it will remain serviceable at least 1 week at ordinary room temperature, if not exposed to direct sunlight. Put the others on ice where they will remain good for 4 to 6 weeks.

As an activating phosphate solution for their urease, Folin and Wu recommend a solution containing 140 gm. of sodium pyrophosphate (U.S.P.) and 20 gm. of glacial phosphoric acid per liter. Two drops are used to activate 1 c.c. of the alcoholic urease solution.

If this change does not occur, the coagulation is incomplete due probably to too much oxalate. In such a case 5 per cent sulfuric acid may be added, a drop at a time, shaking between each addition until the coagulation is complete. If the mixture is now carefully poured upon the double portion of a filter paper just large enough to hold the mixture the filtrate will usually come through perfectly clear, if not, the first portion may be returned to the filter.

Into the left-hand tube of the colorimeter are pipetted 5 c.c. of ammonium sulfate or ammonium chloride solution, containing 0.1 mg. of nitrogen. Two or three c.c. of modified Nessler's solution are now diluted about five times with distilled water to the 10 c.c. mark. This gives a standard 1 mg. N to 100 c.c. in strength. If 0.5 mg. to 100 c.c. dilution is desired, as is generally the case with normal blood, dilute to 20 c.c. with distilled water.

Five c.c. of the blood filtrate are pipetted into the right-hand tube of the instrument and 3-4 c.c. of the diluted Nessler's solution added. (This should be done nearly simultaneously with the addition of the Nessler's to the standard.) The solution should be perfectly clear. Dilution is now made with distilled water, inverting after each addition, until the depth of color is identical with the standard (1 or 0.5 mg. to 100).

(In case one encounters trouble with this method, i. e., turbidity or a yellowish-green color in the unknown which cannot be satisfactorily matched with the standard, it is better to employ the aeration procedure given on the preceding page. After making the aerated acid solution up to some definite volume such as 25 c.c., 5 or 10 c.c. may be pipetted into the right hand tube of the colorimeter and nesslerization then carried out as described below.)

Calculation.—For the calculation of the urea nitrogen, the following formula may be employed in which "S" represents the standard and "R" the dilution of the unknown: $\frac{S}{100} \times R \times 200$ = mg. urea N per 100 c.c. of blood. Since the 5 c.c. of the filtrate employed are the equivalent of 0.5 c.c. of blood, it is obviously necessary to multiply by 200 to obtain the mg. of urea nitrogen per 100 c.c. of blood. With the 0.5 mg. standard and a dilution of 15 c.c. for the unknown the formula would work out as follows: $\frac{0.5}{100} \times 15 \times 200 = 15$ mg. urea N. That is, with this scheme, the dilution of the unknown gives the mg. urea nitrogen directly.

Estimation of Urea in the Saliva

It has long been recognized that urea is a very readily diffusible substance, easily passing through the various membranes in the body. For this reason urea is quite evenly distributed in the various body fluids, as shown by Marshall and Davis.[35] Many analyses are available showing that the urea concentration of the spinal fluid is only slightly less than that of the blood. Myers and Fine[36] found it to average 88 per cent of the blood. Quite recently Schmitz[37] has compared the urea content of the blood and saliva and found the saliva to average 89.4 per cent of the blood.

Obviously salivary urea determinations may be employed in determining the functional activity of the kidneys when for any reason it is impracticable to obtain blood specimens. Schmitz[38] has given the following simple method of estimating the salivary urea.

Method.—A small amount of saliva is collected under the stimulus of paraffin chewing and filtered. Into a test tube graduated at 15 and 20 c.c. (sugar tube) are then introduced 2 c.c. of the filtered saliva, 2 drops of molecular phosphate, and 1 c.c. of a 5 per cent urease solution. The tube is incubated in a beaker of water at 50° C. for 15 minutes. At the end of this time distilled water is added to the 15 c.c. mark, 2 c.c. of dialyzed iron (Merck's 5 per cent Fe_2O_3), 1 c.c. of a 20 per cent sodium sulfate solution, and distilled water to the 20 c.c. mark. After the mixture has been shaken vigorously, it is poured upon a folded

filter. Ten c.c. of the filtrate (equal to 1 c.c. of saliva) are now pipetted into a graduated cylinder and 5 to 10 c.c. of dilute Nessler's solution, depending on the content of nitrogen, are dumped in at once while the solution in the cylinder is rotated until the maximum color development is obtained. If a large amount of urea is present, sufficient distilled water is added until the color of the unknown approximates the color intensity of the standard. The colors are matched immediately in the colorimeter.

If the test tube colorimeter is to be employed, 5 c.c. of the filtrate (equivalent of 0.5 c.c. of saliva) should be taken.

The calculations are the same as those described for blood.

The determination may obviously be carried out by the aeration procedure, which is probably to be preferred, but the procedure described above is much simpler and gives results which agree closely with those obtained by the aeration method.

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CHAPTER III

URIC ACID

SEVENTY-FIVE years ago Sir A. B. Garrod[1] put the subject of the uric acid content of the blood on a definite basis when he identified this substance in the blood of patients suffering from gout, and showed that whereas uric acid was normally present in blood only in traces, it was definitely increased, not only in gout, but also in certain cases of nephritis. He further showed that there is no increase in the blood uric acid in rheumatism, such as is found in gout, and used this as a point of differential diagnosis. No noteworthy advance in this subject was made until Folin and Denis[2] in 1913 introduced their simple colorimetric method for the estimation of this interesting substance. As originally carried out this method was not entirely accurate, but with the modifications since introduced, the method now leaves little to be desired from the standpoint of simplicity and accuracy.

Before considering the results which have been obtained with present methods, further reference should be made to the very interesting work of Garrod, since the general harmony of his conclusions with current views is surprising, when one considers the methods which were then available. It has been suggested that he drew upon his imagination for some of his results, but the correctness of his deductions and the quantitative data which he gives do not support this. This criticism probably originated from the fact that in his later clinical work he endeavored to gauge the amount of uric acid present in the blood by his famous thread test ("uric acid thread experiment").[3] This test was checked against tests where known amounts of uric acid had been employed. In his earlier work, however, the uric acid (or urates) obtained from a given amount of blood, 65 c.c. (1000 grains) was weighed. Such figures as the following recalculated as mg. per 100 c.c. were obtained: in gout, 5, 5, 2.5, 4.5, 3.0 and 17.5; in rheumatism, trace in four and negative in fifth case; in albuminuria, 0.5, 1.2 and 2.7 mg.; and in headache cases, 0.7, trace and 1.0 mg. Sheep and pigeon blood yielded negative results.

The method employed[1] was to evaporate the serum to dryness in thin layers in a water-bath. It was then powdered and treated with rectified spirits, boiled for about 10 to 15 minutes and again treated in the same way. After again washing with spirits, the dried serum was exhausted by means of boiling distilled water, the operation being repeated two or three times, and the watery solutions mixed. The concentrated watery solution was allowed to stand for some hours (forty-eight), when on examination, innumerable tufts of crystals were found deposited on the sides of the vessel, and the surface of the liquid. The crystals were collected, washed with alcohol, and weighed. "These crystals were proved to consist of urate of soda; for crystallized uric acid could be produced from them, and they left an alkaline ash, soluble in water and not consisting of potash."

It is of interest to note that in connection with his later determinations, Garrod writes:[1] "With regard to the weights which have been given, I may observe that in the earlier determinations they doubtless were below the real quantities, a circumstance which arose from the watery solutions of the serum not being sufficiently concentrated, and from sufficient time not being allowed for the deposition of the uric acid. In the experiments now made, I do not collect the crystals until forty-eight hours have elapsed. Such slight errors are, however, unavoidable in new investigations on any subject."

In the course of discussions in his several papers and book, Garrod makes many very interesting and pertinent remarks, a few of which will be quoted: "I may remark, that during evaporation of the serum of blood in albuminuria, a peculiar *odor* of urine was frequently detected; this was not observed in healthy serum, or in that taken from gout or rheumatic patients. Some of the coloring matters of the urine seemed, however, to be thrown down with the uric acid in all cases. * * * The results of these experiments on the condition of the blood and urine prove that uric acid is not a product of the action of the kidneys, as is frequently supposed, but that it is merely excreted from the system by these organs. * * * It appears also probable that as, in albuminuria, the 'urea-excreting function' being chiefly impaired, we find a vicarious discharge of this body in the dropsical effusions; so in gout, the 'uric acid-excreting-function' being defective, the chalk-like deposits are produced, by a similar vicarious discharge of urate of soda. * * * Gout would thus appear partly to depend on a loss of power (temporary or permanent) of this 'uric acid-excreting-function' of the kidneys; the premonitory symptoms, and those also which constitute the paroxysm, arising from an excess of this acid in the blood, and from the effort to expel the 'materies morbi' from the system. * * * In conclusion, as we have found that the blood in every patient suffering from genuine gout, contained an abnormal amount of uric acid, and that in acute rheumatism such was not the condition of this fluid; and again, that in all cases which could be traced up to gout (although the symptoms exhibited at the time might not be very characteristic), uric acid was present, whereas it was absent in those cases where no such phenomena could be found, I think we shall in future be justified in considering the condition of the blood as not only most important, but even a *pathognomonic* sign, and one more to be depended on than any of the other symptoms taken separately." In studying the therapeutic action of colchicum Garrod[4] noted no increase in the uric acid output or influence on urea or other solids of the urine, and observed that this drug did not act as a diuretic in all cases, a diminished output of urine sometimes being noted. He did not confine his attention entirely to uric acid for in a study of blood and effused fluids he writes:[5] "Lastly, these effused fluids may be employed, not only to ascertain the existence of uric acid, but likewise of other principles, as urea and sugar, which are contained and can be detected in them."

As noted above, little advance was made from the time of Garrod until Folin and Denis reported their first results[6] on the uric acid content of human blood. In a series of unselected cases they found between 1 and 3 mg. to 100 c.c., the average being close to 2 mg., while in gout their figures varied between 3.5 and 5.5 mg. Somewhat similar increases were observed in lead poisoning and leucemia. Practically no elevation of the uric acid was noted in a series of 11 nephritic bloods with only moderate nitrogen retention, but later they reported data[7] on cases of advanced nephritis in some of which very high values were obtained, up to 10 mg. These latter observations were confirmed by Myers and Fine,[8] who noted very high figures for uric acid in several cases of terminal interstitial nephritis. In one case the uric acid reached the enormous figure of 27 mg. shortly before death (see Table I), while in several cases figures as high as 15 mg. were observed, values much higher than any noted in gout. Czoniczer[9] believes these high uric acid values fluctuate with the uremic symptoms.

The figures which are now regarded as normal for the blood uric acid differ very little from those originally reported by Folin and Denis. Although healthy adults most often yield values between 2 and 3 mg. per 100 c.c. of blood, figures as low as 1 mg. and as high as 3.8 mg. may be encountered in strictly normal individuals, the differences probably depending in part upon dietary factors. High blood uric acids must obviously depend upon either an increased

formation or a decreased elimination. In leucemia the first factor accounts for the increase, but high uric acids in most other conditions find a probable explanation on the latter basis. Among these may be mentioned gout, nephritis, acute and chronic (but not parenchymatous), eclampsia, arterial hypertension, lead poisoning, bichloride poisoning, malignancy, acute infections, especially pneumonia, and apparently some cases of nongouty arthritis. Miscellaneous cases illustrating the uric acid findings in most of these conditions are given in Table IV of the preceding chapter, and in Table II here. Sedgwick and Kingsbury[10] have made the interesting observation that the blood uric acid is high during the first three or four days of life, in harmony with the high uric acid excretion during that period.

Nephritis

It is perfectly logical to expect that high values for the blood uric acid would be found in the last stages of chronic interstitial nephritis, with the consequent accumulation of all the waste products of nitrogenous metabolism. Comparative figures on the blood and urine in one of our first cases[8] are given in Table I. The blood uric acid findings in this case are higher than in any case

TABLE I

COMPARATIVE BLOOD AND URINE FINDINGS IN CHRONIC INTERSTITIAL NEPHRITIS

I. D., female, aged 17

DATE 1914-15	BLOOD ANALYSES MG. TO 100 C.C.				DATE 1914-15	URINE ANALYSES DAILY AVERAGES IN GMS.		
	Nonprotein N	Urea N	Uric Acid	Creatinine		Total N	Uric Acid	Creatinine
Dec. 10	181	139	6.8	10.0	Dec. 18-19	5.57	0.14	0.37
Dec. 21	199	134	12.5	14.5	Dec. 20-23	3.65	0.07	0.29
Dec. 26	244	151	15.4	17.7	Dec. 23-26	3.64	0.16	0.17
Dec. 30	267	170	21.0	16.1	Dec. 26-30	2.91	0.13	0.13
Jan. 4	297	208	27.0	20.0	Dec. 30 to Jan. 3	1.75	0.09	0.15

we have since studied or any elsewhere reported. Indeed, we might have had some doubts about the last findings recorded, were it not that some of the observations were checked in another laboratory. That the retention of uric acid in nephritis results in a fairly even distribution of this substance in the various body tissues has been shown by Fine[11] in tissues obtained at autopsy. The distribution, however, is not quite as uniform as in the case of the urea or even the creatinine, a fact which might be expected from their physical properties.

In 1916 Myers, Fine and Lough[12] called attention to the fact that very high figures for uric acid may be noted, not only in cases of advanced interstitial nephritis, but also in the very early stage of the disease, before a retention of either the urea or creatinine had taken place. It was suggested that when symptoms of gout were absent, a high blood uric acid might be a valuable early diagnostic sign of nephritis, possibly earlier evidence of renal impairment of an interstitial type than the classic tests of proteinuria and cylindruria. Subsequently, Baumann, Hansmann, Davis and Stevens[13] took up a study of this

question and concluded: "It follows from the above that the uric acid concentration of the blood is a delicate, if not the most delicate, index of renal function at our disposal." More recently Upham and Higley[14] have taken up the renal concentration power for uric acid. It was pointed out by Myers and Fine[15] that under normal conditions the kidney concentrates the uric acid only about twenty times, whereas in the case of urea and creatinine the figures were about 80 and 100, respectively. Upham and Higley have found that in cases free from clinical symptoms of nephritis the concentration power for uric acid was 20 or over, while in cases showing clinical symptoms diagnostic of nephritis, it was 14 or below. In another group of cases showing symptoms suggestive but not diagnostic of nephritis the concentration figure was 18.4 or below.

TABLE II

ILLUSTRATIVE URIC ACID FINDINGS IN NEPHRITIS, LEUCEMIA AND GOUT

CASE	AGE	SEX	DATE	BLOOD ANALYSES MG. TO 100 C.C.			CLINICAL DIAGNOSIS, REMARKS
				Uric Acid	Urea N	Creatinine	
1. H. B.	55	♂	3/28/16	6.1	16	2.8	Chronic diffuse nephritis; hypertension.
2. B. D.	25	♀	3/15/16	9.6	19	2.4	Chronic diffuse nephritis; hypertension and edema.
3. D. S.	56	♂	6/ 7/15	7.1	16	2.0	Chronic interstitial nephritis.
			7/21/15	6.6	24	3.3	
			9/28/15	6.3	18	2.1	
4. J. J.	65	♂	8/11/15	9.5	25	2.5	Chronic interstitial nephritis; died four years later.
			9/25/15	8.0	37	2.7	
			1/14/16	5.0	37	3.9	
5. M. C.	11	♂	6/27/16	8.0	36	2.7	Chronic diffuse nephritis; improvement.
			7/21/16	6.3	53	2.9	
6. W. C.	49	♂	1/15/16	9.5	44	3.5	Acute nephritis; acidosis; recovery.
			1/28/16	2.5	19	1.9	
7. F. F.	8	♂	12/18/17	11.4	106	6.1	Acute nephritis; suppression of urine; recovery.
			12/21/17	11.2	93	5.9	
			12/27/17	5.0	21	4.2	
8. G. McK.	21	♀	12/ 4/19	9.3	48	13.4	Terminal chronic interstitial nephritis.
9. C. P.	37	♂	3/19/20	7.7	111	21.7	Terminal chronic interstitial nephritis.
10. J. P.	62	♂	4/ 4/16	3.4	28	3.0	Chronic parenchymatous nephritis; died.
11. M. E.	27	♀	4/17/20	11.0	13	2.5	Eclampsia; severe convulsions; died.
12. E. P.	20	♀	12/20/20	6.9	6	2.2	Eclampsia; severe convulsions.
13. S. R.	13	♂	8/15/15	10.0	17	2.4	Lymphatic leucemia; died.
14. H. L.	51	♂	3/25/16	7.8	12	2.9	Typical gout for 22 years; tophi on ears; severe pains in joints, particularly right big toe; diet purine-free until after second test.
			4/14/16	6.4	11	2.2	
			5/ 5/16	8.2	15	2.3	
			5/10/16	8.1	15	1.5	

An idea of the uric acid findings in illustrative cases of nephritis may be obtained from Table II. The first two cases show that it is possible to have very high figures for uric acid without any very definite retention of urea. At a later stage in the disease the urea retention becomes definite as is seen in Cases 3, 4, and 5. Acute nephritis may influence not only the uric acid but also the urea and to some extent the creatinine. This is well illustrated by Cases 6 and 7, the first of which showed pronounced acidosis. Note the marked fall in the uric acid following improvement in this case. The nitrogen retention in Case 7 is the most pronounced we have observed with ultimate recovery. The figures on Cases 8 and 9 were obtained in the last stages of the disease, and show a retention of the urea and creatinine as well as of the uric acid. It is of interest regarding the uric acid that early in the disease the values observed may be somewhat higher (7 to 8 mg.) than at a later stage (5 to 6 mg.), due possibly to dietary restrictions, although during the last days of life the amount may be very markedly increased. In parenchymatous nephritis there is very little retention of uric acid. In eclampsia it will be noted that high figures are encountered for uric acid with normal or subnormal urea findings. The high uric acid values are regarded as indicating a slight impairment of renal function (See page 40).

Gout and Arthritis

Owing to the fact that the tophi found in gout have long been recognized to contain deposits of sodium urate, it is quite natural that the uric acid content of the blood in this condition should possess a special interest. Following the investigations of Folin and Denis a number of different workers took up a study of this question, among whom may be mentioned McLester,[16] Pratt,[17] Fine and Chace,[18] and Daniels and McCrudden.[19] Subsequently Folin and Denis[20] and Fine[21] further discussed this question, while recently Pratt in association with McClure,[22] and later McClure[23, 24] have given considerable attention to this subject. McClure's last paper gives an excellent presentation of his own work. In a recent review Pratt[25] gives an excellent discussion of gout. Although the method originally described by Folin and Denis[2] yielded fairly satisfactory results when carefully executed, many figures are recorded for the uric acid content of the blood in gout which are open to question. It is possible by dietary restrictions in the purine-containing foods to lower the blood uric acid in gout, but this would scarcely account for the low results reported by some observers, notably those of Daniels and McCrudden. These investigators reported two cases of gout in women whose blood uric acids were quite normal, although from the case histories given it would scarcely appear that a definite diagnosis had been established. Typical cases of gout generally show comparatively high figures for the blood uric acid, although the figures are not as high as those which may be encountered in chronic interstitial nephritis. Furthermore the uric acid tends to remain high in gout in spite of dietetic measures.

From the normal variations of from 2 to 3 mg. to 100 c.c. of blood, the uric acid may increase to as much as from 4 to 9 mg. in gout, but it does not follow that these uric acid accumulations are infallible signs of gout, since, as noted above, similar uric acid figures may be found in nephritis. This

TABLE III

BLOOD PICTURES IN GOUT AND EARLY INTERSTITIAL NEPHRITIS*

CASE	DATE 1915-1916	AGE	SEX	DIAGNOSIS		URIC ACID, MG. PER 100 C.C.	UREA N, MG. PER 100 C.C.	CREATININE, MG. PER 100 C.C.	PHTHALEIN 2 HRS. PER CENT.	SYSTOLIC BLOOD PRESSURE	URINE	
											PROTEIN	CASTS
M. K.	9/ 3	49	♀			9.5	13	1.1	48	230	+	−
T. B.	10/ 5	57	♂			8.4	12	2.2	35	164	−	+
H. L.	3/24	51	♂	Typical gout.....		7.8	12	2.9	59	120	−	−
L. J.	10/ 6	43	♂			7.2	17	2.4	..	200	−	−
C. P.	10/ 6	45	♂			6.8	14	1.7	..	...	....	....
B. D.	3/31	25	♀	Miscellaneous	Slight edema.....	7.7	20	2.6	45	168	+	−
D. S.	6/ 7	56	♂	cases	Hypertension	6.7	19	2.5	26	185	−	+
E. V.	3/20	14	♀	Showing some	Endocarditis	6.3	12	3.6	32	120	++	+
H. B.	3/28	55	♂	evidence of	Incipient nephritis...	6.1	16	2.8	57	120	−	+
H. J.	3/23	50	♂	early	Diabetes	6.0	18	2.9	36	140	+	+
G. C.	3/14	40	♀	interstitial	Incipient nephritis...	5.5	15	2.1	46	147	−	+
M. S.	3/22	46	♀	nephritis	Gastritis	5.0	12	2.5	52	190	+	+

*Taken from Fine.[21]

point is well brought out in the table on page 55 taken from Fine. A very close scrutiny of Table III does disclose a tendency for the urea and creatinine concentrations of Group 2 to rise above those of Group 1, which are essentially normal, but the differences are slight. Myers and Fine[12] have stated, "The blood pictures in early interstitial nephritis and gout are strikingly similar, particularly as regards the increase in uric acid. In view of the other clinical symptoms in common, it would seem that this similarity must be more than accidental." Fine[21] has raised these questions: "1. Is gout merely a stage in the development of interstitial nephritis, whose further progress may be indefinitely delayed? 2. Is early interstitial nephritis merely potential gout, in which the clinical symptoms may or may not appear? 3. Is the uric acid retention of gout due to the specific condition, *gout,* or to a complicating early interstitial nephritis?" McClure[24] has studied the renal function in six cases of gout in which the clinical diagnosis of nephritis was not justifiable. By means of the phthalein test, nonprotein and urea nitrogen and two-hour renal test, some disturbance of renal function was demonstrated in each case, thus strengthening the general argument given above.

Practically, we may conclude that gout is almost invariably associated with an increased uric acid content of the blood and therefore a high blood uric acid may be of considerable diagnostic value in cases of gouty arthritis, in which tophi containing sodium urate are not already present. It should be borne in mind that cases of arthritis may occasionally show an increase in the uric acid, although this is generally associated with an increased urea and nonprotein nitrogen, thus indicating a complicating nephritis. Such cases of arthritis, however, are the exception rather than the rule. In ten typical cases of arthritis which came under our observation during the month of April, 1920, the figures for the blood uric acid ranged from 1.6 to 3.6 mg. to 100 c.c. and all but three cases showed under 3 mg. In a more extended study of the uric acid in arthritis, Chace, Myers and Killian[26] encountered only 2 or 3 cases where the uric acid exceeded 4 mg. per 100 c.c., and in these cases evidence of impaired renal function was present.

Influence of Diet and Drugs upon the Blood Uric Acid

That a purine-free diet will definitely, although not markedly lower the blood uric acid in gout is brought out by the observations on Case 14 in Table II, where in the course of about three weeks the uric acid fell from 7.8 to 6.4 mg., but again rose to 8.2 and 8.1 mg. upon the return to a purine diet. A much more pronounced lowering of the uric acid as the result of dietary restrictions may be noted in the first two cases of Table IV.

It has been recognized for some time that the administration of salicylates and phenylcinchoninic acid (cinchophen) results in an increased excretion of uric acid in the urine. The influence of phenylcinchoninic acid upon uric acid elimination was first observed by Nicolaier and Dohrn[27] and ascribed by them to a stimulation in nuclear metabolism, but later Weintraud[28] advanced the view that this drug exerts a selective action upon the kidneys, by which the uric acid is removed from the blood and tissues. Several years

TABLE IV

INFLUENCE OF DIET AND DRUGS UPON THE BLOOD URIC ACID

CASE	AGE	SEX	DATE	BLOOD ANALYSES MG. TO 100 C.C.			CLINICAL DIAGNOSIS, REMARKS
				Uric Acid	Urea N	Creatinine	
1. P. P.	28	♂	3/14/16	8.0	20	2.7	Gastric ulcer; diet until 3/17 contained broths, but later was purine-free.
			3/17/16	6.3	15	2.4	
			3/28/16	(3.7	20	...	
			4/ 4/16	(3.1	14	1.8	
2. M. S.	46	♀	2/23/16	6.1	12	2.6	Arteriosclerosis, periodic vomiting; diet purine-free from 2/29 until after 3/16; 60 grains of sodium salicylate 3/27 to 4/2, then purine-free diet.
			3/ 7/16	(3.1	12	2.9	
			3/16/16	(3.0	12	2.5	
			3/23/16	5.0	12	2.5	
			3/31/16*	1.7	13	3.4	
			4/ 7/16	2.5	..	...	
			4/18/16	5.0	12	2.3	
3. T. G.	46	♂	6/27/16	6.0	17	2.6	Chronic duodenal ulcer, syphilis; purine-free diet 3 days previous to 1st analysis; 30 grains of a combination of Na salicylate and cinchophen daily for 4 days preceding 2nd analysis.
			7/ 1/16*	3.2	18	1.8	
4. M. G.	58	♂	3/26/20	6.0	29	1.5	Chronic interstitial nephritis, arteriosclerosis; 50 grains of neocinchophen given 3/28 to 4/3.
			3/28/20	5.2	29	2.4	
			3/31/20*	trace	16	2.0	
			4/ 5/20	3.0	24	2.2	
			4/12/20	3.5	18	3.0	
			4/13/20	3.9	15	2.4	
5. M. D.	53	♀	3/16/20	2.8	26	2.6	Neurasthenia, visceroptosis; vegetable diet; neocinchophen 50 grains daily 3/19-22; cinchophen 50 grains daily 4/9-12.
			3/18/20	2.8	22	2.6	
			3/22/20*	trace	13	2.7	
			3/25/20	2.6	20	2.4	
			3/29/20	2.6	19	2.2	
			4/ 9/20	2.8	16	2.4	
			4/12/20*	0.7	13	2.6	
			4/15/20	2.0	17	2.4	
6. J. C.	40	♀	1/ 6/21	7.4	20	3.6	Cardionephritis; 50 grains neocinchophen 1/9-15; diet constant; at rest in bed throughout.
			1/ 8/21	7.1	21	3.1	
			1/12/21*	4.8	15	2.2	
			1/15/21*	trace	14	2.5	
			1/20/21	6.8	23	3.2	
			1/27/21	6.0	26	3.5	
7. G. McK.	21	♀	12/ 4/19	9.3	48	13.4	Chronic interstitial nephritis; patient died 12/30; 25 grains cinchophen daily 12/5-8 with lemonade diet; low protein diet with 25 grains of a mixture of cinchophen and theobromine daily 12/9-11.
			12/ 5/19	10.4	42	16.8	
			12/ 6/19*	5.1	52	12.7	
			12/ 7/19*	8.3	45	15.0	
			12/ 8/19*	8.1	48	14.3	
			12/ 9/19*	5.4	55	12.3	
			12/11/19*	7.5	57	12.1	
			12/13/19	6.1	53	13.0	
8. S. H.	37	♂	5/10/15	7.7	76	10.9	Chronic interstitial nephritis; 45 grains cinchophen on 5/12 and 13, 60 grains on 5/14; patient died 6/28.
			5/15/15*	8.0	64	9.6	
			5/21/15	7.8	53	8.0	

*Specimens taken during period of medication.

later it was shown by Folin and Lyman[29] and Fine and Chace,[18] in particular, that this action is accompanied by a marked drop in the uric acid content of the blood, and later the same was shown to be true of salicylates by Fine and Chace [30] and Denis.[31] As a result of the studies on the blood it became clearly evident that the increased elimination of uric acid after the administration of these drugs was due to increased renal function. The action of phlorhizin and the methylated purines on the kidney is better known but scarcely as remarkable as that of the drugs mentioned. The action of phlorhizin holds little of direct clinical interest, and Christian [32] and his coworkers have recently thrown serious doubts on the therapeutic value of the methylated purines.

It has been assumed that salicylic acid, cinchophen and their derivatives induce an increased output of uric acid in the urine and a decreased concentration in the blood (and tissues) by endowing the renal cells with an increased power for eliminating uric acid. The effect of these drugs begins to be manifest nearly as soon as absorption of the drug has taken place and exerts its maximum influence in about one day. There is some evidence to indicate that the improved kidney function is probably dependent upon an altered circulation in the glomeruli, due to a mild congestion and irritation.

Although the salicylates have been employed in infectious arthritis largely because of their analgesic and antipyretic properties, and cinchophen has been employed in gout because it stimulates uric acid excretion, a closer study of these two classes of compounds has shown that, in general, they possess somewhat the same properties, i, e., the salicylates also increase the elimination of uric acid, and cinchophen possesses analgesic properties. It is a singular fact that drugs possessing such similar therapeutic properties should have come for entirely different reasons, to be used in the treatment of infectious and gouty arthritis. These facts led Chace, Myers and Killian[26] to believe that, possibly after all, these two forms of arthritis might possess something in common. From a study of a large series of cases of infectious arthritis, however, they were unable to discover any relation between the drop in uric acid after the administration of these drugs and the clinical improvement. In conditions with high blood uric acid, such as gout, it would appear more difficult to decide as to which property was the more valuable, the analgesic or the influence on the elimination of uric acid. It seems probable, however, that here as in infectious arthritis their clinical value is closely associated with their analgesic properties. There is some experimental evidence which suggests the possibility that the clinical benefit may be brought about by an improved circulation to the inflamed joints, thus facilitating the interchange of inflammatory and metabolic products. It is well to bear in mind in this connection that colchicum so long employed in the treatment of gout has been recognized since the time of Garrod[4] to have no influence upon the elimination of uric acid.

Attention has already been called to the fact that of the three nitrogenous waste products, uric acid, urea and creatinine, creatinine is the most readily and uric acid the most difficultly eliminated, with urea standing in somewhat of an intermediate position. In harmony with this a uric acid re-

tention may be found in the early stages of nephritis, but creatinine retention only in the terminal stages of the disease. One would therefore expect that drugs, which have a general stimulating effect on the kidney, would affect the excretion of uric acid first, urea next and creatinine last. From the observations of Myers and Killian[33] recorded in Table IV (Cases 4, 5 and 6) such appears to be the case. This would indicate that the action of these drugs is not specific for uric acid. To be sure, the action on the uric acid is by far the most marked, but the term "uric acid eliminant" frequently applied to these drugs is somewhat misleading, since these drugs as pointed out by Myers and Killian also stimulate, to a lesser degree, the elimination of other waste products, as urea, chlorides, creatinine.

Although the action of the salicylates and cinchophen is very similar, cinchophen and neocinchophen, the methyl derivative of its ethyl ester, have an advantage in that they appear to be free from the toxic influences on the kidney inherent in the salicylates. Neocinchophen (obtainable under the name of tolysin) is tasteless and is better tolerated by some patients than cinchophen.

The influence of these drugs upon the blood uric acid is well illustrated in Table IV. Case 2 shows the effect of sodium salicylate and Case 3 a combination of this drug with cinchophen. In Cases 4, 5, and 6 neocinchophen was employed, Cases 4 and 6 showing a high and Case 5 a normal initial uric acid. The control urea nitrogen in all three cases was definitely above normal. In these three cases the uric acid was reduced to a quantity too small to estimate, while the urea nitrogen dropped to normal. Case 6 showed a slight creatinine retention which was favorably influenced by the neocinchophen administration. Cinchophen was subsequently employed in Case 5 with a somewhat less pronounced effect than when neocinchophen was given. As will be noted the discontinuance of the drugs resulted in a complete or partial restoration of the initial figures in all three cases.

Fine and Chace[34] have pointed out that in the last stages of interstitial nephritis cinchophen has little influence on the excretion of uric acid, indicating that the renal cells can no longer be stimulated to increased activity. Cases 7 and 8 illustrate this point, no change in the uric acid being noted in these two cases, even after large doses of cinchophen.

Estimation of Uric Acid

In 1912 Folin and Macallum[35] called attention to the possibilities of the use of phosphotungstic acid in the colorimetric estimation of uric acid, and later in the same year Folin and Denis[2] gave their first description of this estimation as applied to blood. As already pointed out, the results which they first obtained with this method clearly demonstrated the increase in the blood uric acid in gout and lead poisoning[6] and in nephritis.[7] In carrying out these determinations the procedure was to coagulate the blood protein with weak (0.01 N) acetic acid after a 1 to 6 dilution with this solution, filter, wash, evaporate to a very small volume and then precipitate the uric acid with silver lactate, magnesia mixture and ammonia in a small centrifuge tube. (Owing to the presence of

phenols in blood it is necessary with this technic to separate the uric acid from these color reacting substances.) This was then thrown down in the centrifuge and the precipitate decomposed with hydrogen sulfide. After removal of the excess of hydrogen sulfide, the color reaction was then developed with the special phosphotungstic acid reagent and sodium carbonate.

It could hardly have been expected that such a new method would be perfectly satisfactory in every detail, and in 1915 Benedict[36] introduced certain modifications which did much to increase the accuracy and simplicity of the method. Possibly the greatest source of error was in the decomposition of the silver precipitate with hydrogen sulfide, since low results were obtained, unless considerable care was taken in breaking up the precipitate. For this purpose Benedict employed potassium cyanide, which proved to be very satisfactory. Other improvements suggested by Benedict were a more dependable standard uric acid solution, the combined use of the silver lactate, magnesia mixture and ammonia into a single reagent and the use of colloidal iron to remove the last trace of protein after coagulation. For this purpose Myers and Fine[12] employed alumina cream, in preference to the colloidal iron. Although this method[12] was somewhat tedious, various checks led us to believe that it gave perfectly reliable results. In 1919 Folin and Wu,[37] in connection with their system of blood analysis, described a method of estimating uric acid directly in the protein-free filtrate obtained after their tungstic acid precipitation without evaporation of the filtrate. Such a procedure obviously greatly simplified the technic of this estimation. Folin and Wu precipitated the uric acid from the filtrate with a special lactic acid-silver lactate reagent, after which the uric acid was set free from the silver precipitate by a solution of sodium chloride containing hydrochloric acid. In order to secure a permanent standard they made use of sodium sulfite to prevent the oxidation of uric acid. As they noted, the use of this reagent cut down the peculiar intensification of the blue color obtained by the use of cyanide, and thus made the color difficult to read. Although conceding the general accuracy of the results obtained with the Folin-Wu technic, Benedict[38] has pointed out several theoretical and practical objections to the method. During the past year he has described an extremely simple technic whereby the uric acid color reaction is developed directly on the Folin-Wu tungstic acid blood filtrate. This method appears to have been quite generally adopted.

The essential points of departure in Benedict's new procedure are the use of a new uric acid reagent (arsenic phosphotungstic acid), cyanide as the sole alkali and heat in the development of the color. This new reagent, when employed as described by Benedict (given below), yields nearly seven times as much color from a given weight of uric acid, as the old reagent employed according to the old procedure. This reaction appears to be very highly specific for uric acid, and may therefore be carried out directly on the blood filtrate without the preliminary precipitation of the uric acid. According to Benedict, the increased specificity of the new procedure as regards uric acid lies probably chiefly in the reagent employed and only partly in the use of cyanide instead of carbonate for development of alkalinity. He also states that a corresponding, though not so great, increase in color is obtained

with the old phosphotungstic acid reagent through the use of large quantities of cyanide but that the reaction thus obtained is not very specific for uric acid. Folin[39] believes that the secret of success in Benedict's new method is not the new reagent but the use of cyanide as the sole alkali. He has adopted most of Benedict's suggestions, except that he still employs the original Folin-Denis uric acid reagent (see page 152). Benedict[40] has stated that he believes his new reagent preferable, even though its only advantage (and this he does not concede) might be its greater simplicity of preparation.

With the original Folin-Denis method for uric acid 25 c.c. of blood were employed, if this amount could be secured. With the present method, which is very much more accurate, the color reaction is developed on the equivalent of 0.5 c.c. of blood. If uric acid is the only determination made on the tungstic acid filtrate, this calls for the use of 1 to 2 c.c. of blood. It is perfectly possible, however, as Benedict has pointed out, to carry out the determination on as little as 0.2 c.c. of blood (0.1 c.c. of filtrate), an amount readily obtainable by puncture.

Method.[38]—To 2 c.c. of well-mixed oxalated blood in a large test tube or 25 c.c. cylinder, add 14 c.c. of water (7 volumes), 2 c.c. of 10 per cent sodium tungstate* and then 2 c.c. of exactly 2/3 N sulfuric acid while rotating the test tube or cylinder. Shake thoroughly and allow to stand at least 10 to 20 minutes. This tends to insure complete protein precipitation. When the blood is properly coagulated, the color of the coagulum turns from pink to brown. (If this change does not occur, the coagulation is incomplete, due probably to too much oxalate. In such a case, 5 per cent sulfuric acid may be added a drop at a time, shaking between each addition, until the coagulation is complete. Folin and Wu *caution against an excess* of sulfuric acid, since this apparently brings about a precipitation of the uric acid.) If the mixture is now carefully poured upon the double portion of a filter just large enough to hold the mixture, the filtrate will probably come through perfectly clear; if not, the first portion may be returned to the filter. To prevent evaporation, a watch glass may be placed over the top of the funnel. It is convenient to filter into a test-tube or 25 c.c. cylinder.

Pipette 5 c.c. of the water clear filtrate (representing 0.5 c.c. of blood) into a test-tube (18 to 20 mm. in diameter) and add 5 c.c. of water. Into a similar test-tube pipette 5 c.c. of a dilute uric acid standard† (containing 0.02 mg. uric acid) and add 5 c.c. of water. To both standard and unknown now add (from a burette) 4 c.c. of 5 per cent sodium cyanide solution containing 2 c.c. of concentrated ammonia per liter. To each tube then add 1 c.c. of Benedict's arsenic phosphoric acid tungstic acid reagent‡ thus making both tubes up to exactly 15 c.c. in volume.

The contents of each tube should be mixed by one inversion immediately after addition of the reagents, and placed immediately in boiling water, where the tubes should be left

*The purity of the tungstate may need to be tested (for tests see page 145).

†Benedict's Standard Uric Acid Solution.—This is prepared as follows: Dissolve 4.5 gm. pure crystalline hydrogen disodium phosphate and 0.5 gm. dihydrogen sodium phosphate in 200 to 300 c.c. hot water. Filter and make up to about 250 c.c. with hot water. Pour this warm, clear solution on 100 mg. uric acid suspended in a few c.c. of water in 500 c.c. volumetric flask. Agitate until completely dissolved. Add at once exactly 0.7 c.c. glacial acetic acid, make up to 500 c.c., mix and add 5 c.c. chloroform. One c.c. of this solution contains 0.2 mg. uric acid. This solution should be freshly prepared every 2 months.

Benedict's Dilute Uric Acid Standard.—A dilute standard containing 0.02 mg. uric acid in 5 c.c. of solution may be prepared by diluting Benedict's phosphate standard as follows: 10 c.c. of the phosphate standard (containing 2.0 mg. of uric acid) are pipetted into a 500 c.c. volumetric flask, and the flask is about half filled with distilled water. 25 c.c. of dilute hydrochloric acid (1 volume of concentrated acid diluted to 10 volumes with water) are added, and the solution is diluted to 500 c.c. This standard should be freshly prepared once in 2 weeks.

‡Benedict's New Uric Acid Reagent.—This is prepared by dissolving 100 gm. of pure sodium tungstate in about 600 c.c. of water in a liter flask, then adding 50 gm. of pure arsenic pentoxide, followed by 25 c.c. of 85 per cent phosphoric acid and 20 c.c. of concentrated hydrochloric acid. The mixture is boiled for 20 minutes, cooled, and diluted to 1 liter. The reagent appears to keep indefinitely.

for 3 minutes after immersion of the last tube, but the time elapsing between immersion of the first and last tubes should not exceed 1 minute. One should not attempt to run more than five tubes in one series. After 3 to 4 minutes heating the tubes are removed and placed in a large beaker of cold water for 3 minutes and read in a colorimeter against the standard as soon as may be convenient. One should read the solutions within 5 minutes after removing from the cold water, otherwise turbidity may develop.

Where a large number of bloods are to be analyzed, it is best to run not more than four at a time with one standard. This provides for greater uniformity in handling, does not cool the bath down too much, and makes it easy to finish reading before any turbidity may develop.

Calculation.—Employing the standard solution containing 0.02 mg. of uric acid and using 5 c.c. of the 1 to 10 blood filtrate, the calculation for the uric acid content of the original blood is as follows: $\frac{S}{R} \times 4 =$ mg. of uric acid per 100 c.c. of original blood, in which S represents the height of the standard solution in millimeters, and R the reading of the unknown solution. If instead of using 5 c.c. of blood filtrate in the determination, 2.5 or 10 c.c. are employed, the final figure is multiplied or divided by 2 as the case may be.

Estimation of Uric Acid with the Test Tube Colorimeter

The technic described above readily lends itself to use with the test tube colorimeter.

Method.—One c.c. of the well mixed oxalated blood is pipetted into a test tube and 7 c.c. of water added. The precipitation of the proteins is now carried out according to the procedure of Folin and Wu, as described above, 1 c.c. each of the sodium tungstate and 2/3 N sulfuric acid being added.

In case it is necessary to add extra sulfuric acid, this should be done carefully as an excess may lead to a precipitation of the uric acid.

Into the left hand tube of the colorimeter are pipetted 3 c.c. of Benedict's dilute uric acid standard (containing 0.012 mg. uric acid), while 2, 3, 4 or 5 c.c. of the tungstic acid blood filtrate are similarly transferred to the right hand tube of the instrument. The amount of uric acid in the unknown should correspond as closely as possible with the standard; for normal bloods 4 c.c. is about the right amount. To both tubes are now added (from a burette) 4 c.c. of 5 per cent sodium cyanide solution containing 2 c.c. of concentrated ammonia per liter and 1 c.c. of Benedict's arsenic phosphoric acid tungstic acid reagent.

The contents of both tubes should be mixed by inversion immediately after addition of the reagents, and at once placed in a beaker of boiling water, where the tubes should be left for 3 minutes after immersion. An appreciable amount of time is required for dilution and one can best carry out only one determination at a time with this simple clinical instrument. If the test is run through without unnecessary delay, there should be no trouble from turbidity. After 3 to 4 minutes' heating, the tubes are removed and placed in a large beaker of cold water for 3 minutes. As soon as this time has elapsed the tube containing the standard is diluted to the 10 c.c. mark with the aid of the diluting pipette, mixed by inversion and then placed in the left hand compartment of the instrument. The tube containing the unknown is now placed in the right hand compartment, to obtain an idea of the dilution required. Distilled water is added to the unknown with the diluting pipette, inverting after each addition until the intensity of color of both tubes is the same. Just before the end point is reached the water should be added a drop or two at a time.

Calculation.—To calculate the mg. of the uric acid per 100 c.c. of blood, the following formula may be used, in which S represents the strength of the standard (equivalent of 0.12 mg. to 100 c.c.), D the dilution in c.c. of unknown required to match the standard, and B the amount of blood employed in c.c.: $\frac{S \times D}{B} =$ mg. uric acid per 100 c.c. of blood.

If, for example, 4 c.c. of filtrate are used, the equivalent of 0.4 c.c. of blood with a standard containing 0.012 mg. diluted to 10 c.c. (the equivalent of 0.12 mg. to 100 c.c.), and the dilution of the unknown required to match the standard is to 12.3 c.c., the formula will work out as follows:

$$\frac{0.12 \times 12.3}{0.4} = 3.7 \text{ mg. to } 100 \text{ c.c.}$$

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CHAPTER IV

CREATININE

UNTIL the advent of Folin's colorimetric method for the estimation of creatinine in urine in 1904,[1] we possessed no reliable information regarding this interesting nitrogenous waste product, which in point of quantity is second only to urea. Folin[2] was the first to show that the amount of creatinine excreted in the urine by a normal individual on a meat-free diet is quite independent of either the amount of protein in the food or of the total nitrogen in the urine, the amount excreted from day to day being practically constant for each individual, thus pointing conclusively to its endogenous origin. He further noted that the fatter the subject, the less creatinine is excreted per kilo of body weight and concluded from this that the amount of creatinine excreted depends primarily upon the mass of active protoplasmic tissue, or, as Shaffer[3] has expressed it, "Creatinine is derived from some special process in normal metabolism taking place largely, if not wholly, in the muscles, and upon the intensity of this process appears to depend the muscular efficiency of the individual." Creatinine is the anhydride of creatine, the chief nonprotein nitrogenous constituent of the muscle tissue of vertebrate animals. That the creatinine of the urine has its origin in the creatine of the muscle would seem obvious on *a priori* grounds, but a definite proof of this hypothesis has been beset with many difficulties. The older observers stated that both administered creatine and creatinine reappeared in the urine as creatinine. When Folin[4] first reinvestigated this question with accurate methods and pure creatine and creatinine, he found that 80 per cent of the administered creatinine did reappear as creatinine, but that when creatine was given in moderate amounts (1 gram to man) it not only failed to reappear as creatinine, but completely disappeared. From this Folin quite naturally concluded that creatine and creatinine were relatively independent in metabolism.

In 1913 Myers and Fine[5] called attention to the fact that the creatine content of the muscle of a given species of animals was very constant (obviously also that of a given animal) and suggested this as a possible basis of the constancy in the daily elimination of creatinine first noted by Folin. Later they pointed out that the creatinine content of muscle was greater than that of any other tissue, and also that in autolysis experiments with muscle tissue the creatine (and any added creatine) was converted to creatinine at a constant rate of about 2 per cent daily, which is just about the normal ratio between the muscle creatine and urinary creatinine. They also found that, when creatine was administered to man or animals, there was a slight conversion to creatinine which corresponds well with the above figure. These facts all go to support the view that creatinine is formed in the muscle tissue from creatine, and at a very constant rate, although no explanation of the physiological significance of this

transformation can as yet be offered. Excepting possibly the kidney, the muscle normally contains more creatinine than any other body tissue and is followed by the blood, which indicates that after its formation in the muscle the creatinine is carried to the kidney by the blood stream. The only exception to this statement is found in "uremia" where the creatinine content of the blood may slightly exceed that of the muscle.[6]

Folin and Denis[7] were the first to present any very extensive data on the creatinine content of the blood, although almost simultaneously Neubauer[8] reported an observation on a case of "uremia," while Myers and Fine[9] presented several analyses on two cases of nephritis showing marked retention of creatinine. Folin and Denis gave observations on nine cases of "uremia" and were the only observers to give figures for normal human bloods. Shaffer had begun a study of this question some years previously, but unfortunately did not give his protocols[10] until after the appearance of the paper by Folin and Denis. In a preliminary report in 1909 Shaffer and Reinoso[11] stated that using methods which they believed allowed no conversion of creatine to creatinine, they found between 1 and 6 mg. of creatinine per 100 grams of fresh dog muscle, and about 1 mg. per 100 c.c. of serum. Later Myers and Fine took up a study of the creatinine content of muscle tissue and reported some preliminary observations,[12] which confirmed the work of Shaffer and Reinoso. They had described their method[13] but not given their protocols[14] prior to the publication of Folin's simple method[15] for the estimation of creatinine in body fluids and tissues. Essentially the same range of figures was found with the two methods, the findings for various animals and man ranging roughly from 3 to 9 mg. per 100 grams of muscle, excepting instances of impaired renal function.

Creatinine Content of the Blood

For perfectly normal individuals the creatinine of the blood amounts to 1 to 2 mg. per 100 c.c., the findings for the strictly normal being nearer 1 than 2 mg. As soon as one passes to hospital patients, however, higher values are found. Although the great majority of cases without renal involvement show creatinine figures on the whole blood below 2.5 mg. per 100 c.c., occasionally figures as high as 3.5 mg. are encountered that are not readily explained. It may be noted, however, that a slight retention of creatinine (figures between 3 and 4 mg.) occurs in syphilis, certain heart conditions, sometimes in fevers, and in some cases of advanced diabetes. Creatinine figures above 3.5 mg. are almost invariably accompanied by an appreciable urea retention and this is generally true of those above 3 mg. Many of the cases below 4 mg. show improvement, but with over 4 mg. the reverse is the case. It would appear from this that an appreciable retention of creatinine, *i.e.*, over 4 mg., does not occur until the activity of the kidneys is greatly impaired. That such should be the case is quite natural to expect, since creatinine is normally the most readily eliminated of the three nitrogenous waste products, uric acid, urea, and creatinine.

In a recent contribution Behre and Benedict[16] present evidence to show that creatinine does not exist in normal blood, although their data are not quite as definite in the case of nephritic bloods. The various facts upon which they make this deduction are, (1) that creatinine can be removed from

blood by bone black, but bone black does not affect the chromogenic substance present in blood filtrates, which react with picric acid and sodium hydroxide, (2) that a substance present in picric acid blood filtrates gives a good reaction with sodium carbonate, and this reaction is increased in bloods showing an abnormally high "creatinine" content, whereas pure creatinine in picric acid is only slightly affected by carbonates, (3) that creatinine is readily destroyed by heating in alkaline solution, but the chromogenic substance present in blood is not appreciably affected, (4) that kaolin completely removes small amounts of creatinine from blood, but does not affect the picric acid-alkali reacting substance. With bloods showing more than 4 mg. "creatinine" per 100 c.c., the kaolin removes only a portion of the chromogenic substance, and Behre and Benedict state that they would be inclined to the view that true creatinine accumulates in the blood after impairment of the kidney function, if it were not for the fact (5) that they have been unable to isolate creatinine from nephritic bloods supposedly high in their creatinine content, although added creatinine has been readily isolated. They conclude: "Our findings that creatinine does not exist in blood in detectable quantities need not, of course, raise any question as to the value of the determination of the chromogenic substance for clinical or other purposes."

In the light of the above observations, how is the presence of creatinine in the urine to be explained? Behre and Benedict suggest that the kidney may be able to concentrate the creatinine from extremely dilute solutions or produce the creatinine from some common precursor substance in the blood. They are inclined to the latter view and suggest creatine as the probable precursor. They have presented real evidence of the existence of creatine in blood, but creatine has not ordinarily been looked upon as a waste product, as would be called for in their hypothesis. Furthermore, this view is not in harmony with what we know regarding the transformation of creatine to creatinine.

These observations of Behre and Benedict may be reconciled with our present views by supposing that the chromogenic substance present in blood is closely related to creatinine and readily converted to creatinine in the kidney. What the nature of this chromogenic substance may be it is not possible to suggest at present. That closely related compounds may give the color reaction, however, is evident from the fact that the ethyl ester of creatine gives the same color reactions as creatinine. There is no reason to believe that this compound is present in the body, but it seems difficult to explain the increase in the chromogenic substance in nephritic bloods on any other ground than impaired excretion of creatinine.

That the taking of creatinine by mouth will lead to an increase in the creatinine reaction of the blood is evident from the observations of Berglund[17] given in Table I.

The normal blood creatinine of the subject of the experiment was 1 mg., but after single doses of 1, 5 and 10 gms. creatinine the blood creatinine rose to 2.17, 4.75 and 7.9 mg. respectively. These observations as Berglund has pointed out, do not give any definite information about the presence of creatinine in

TABLE I

THE INCREASE OF THE BLOOD CREATININE, ON H. B., AGED 33 YEARS, WEIGHT 90 KG., AFTER TAKING CREATININE BY MOUTH*

TIME, 1920	EXPERIMENTAL CONDITION	BLOOD CREATININE
		mg. per 100 c.c.
July 2	Creatinine-free diet	1.04
July 10	After 1 gm. creatinine	2.27
July 13	After 3 gm. creatinine	2.5
July 13	After 3 gm. creatinine, in 2 doses	2.12
July 17	After 5 gm. creatinine	4.75
July 21	After 10 gm. creatinine	7.9
July 21	After 10 gm. creatinine, in 2 doses	6.15
July 22	After 10 gm. creatinine, in 3 doses	2.14

*Taken from Berglund.[17]

normal blood, but they do show that the blood creatinine may be readily influenced by the taking of creatinine. Furthermore, as shown in Table I of the preceding chapter, the increase in the blood creatinine in chronic nephritis goes hand in hand with a decreased renal excretion.

Creatine Content of the Blood

Normally the creatine content of the blood amounts to from 3 to 7 mg. per 100 c.c., although the amount may be greatly increased in the last stages of nephritis along with other nonprotein nitrogenous substances. Creatine appears to be present in the cells in much greater concentration than in the plasma, as might be expected from its content in other body tissues. Its estimation in blood has not been shown to possess any great practical importance, although as just indicated it is considerably increased in the terminal stages of nephritis. The relationship of the creatine of the plasma to the excretion of creatine in the urine is of some interest. Table II taken from Berglund[17] well illustrates this. The taking of pure creatine by mouth

TABLE II

INCREASE (DELAYED) OF PLASMA CREATINE AND CORRESPONDING CREATINE OUTPUT CAUSED BY TAKING CREATINE. SUBJECT, W., MALE, AGED 24, WT. 70 KG. (AFTER BERGLUND[17])

TIME MAY 11, 1921	WHOLE BLOOD PER 100 C.C.		PLASMA PER 100 C.C.		CORPUSCLES PER 100 C.C.		URINE PER HOUR		
	CREATINE	CREATININE	CREATINE	CREATININE	CREATINE	CREATININE	VOLUME	CREATINE	CREATININE
	mg.	mg.	mg.	mg.	mg.	mg.	c.c.	mg.	mg.
10:55 a. m.	3.4	1.1	1.9	1.1	4.9	1.1	42	0	73
11:00 a. m.*	...	...	...	...	...	...	..	..	..
11:52 a. m.	3.7	1.0	1.9	1.1	5.7	1.0	..	..	..
12:00 m.	...	...	...	...	...	...	83	0	81
1:08 p. m.	3.4	1.1	1.9	1.1	5.1	1.1	..	..	..
1:15 p. m.	...	...	...	...	...	...	288	0	86
3:00 p. m.	3.8	1.2	2.3	1.2	5.5	1.2	..	..	..
3:05 p. m.	...	...	...	...	...	...	44	29	82
4:35 p. m.	3.8	1.1	2.6	1.2	5.3	1.1	..	..	..
4:44 p. m.	...	...	...	...	...	...	33	63	87
5:50 p. m.	3.8	1.1	1.9	1.1	6.4	1.1	..	..	..
6:00 p. m.	...	...	...	...	...	...	28	35	77

*Took 4 gm. crystalline creatine + 200 gm. glucose + 600 c.c. water. Subject fasting; urinated at 9:01 a. m.

in doses of 4 gm. is sooner or later followed by an increase in the creatine figures of the plasma and the smallest appreciable increase of the creatine in the plasma is accompanied by excretion of creatine in the urine.

TABLE III

THE PROGNOSTIC VALUE OF THE CREATININE OF THE BLOOD IN NEPHRITIS*

CASE	AGE	BLOOD ANALYSES MG. TO 100 C.C.		TIME UNDER OBSERVATION	OUTCOME	CASE	AGE	BLOOD ANALYSES MG. TO 100 C.C.		TIME UNDER OBSERVATION	OUTCOME
		Creatinine	Urea N					Creatinine	Urea N		
1	25	33.3	240	1 mo.	Died	44	64	9.7	70	5 mos.	Died
2	39	28.6	186	3 wks.	"	45	30	9.5	140	2 mos.	"
3	53	22.5	106	2 wks.	"	46	51	9.5	89	6 mos.	"
4	37	22.2	262	5 wks.	"	47	69	9.5	89	2 wks.	"
5	34	20.5	152	2 mos.	"	48	..	9.2	54	2 days	"
6	17	20.0	209	1 mo.	"	49	56	9.1	224	2 wks.	"
7	43	20.0	162	4 days	"	50	43	8.8	55	3 wks.	"
8	25	20.0	108	3 wks.	"	51	27	8.3	59	3 mos.	"
9	53	19.8	114	2 wks.	"	52	40	8.3	75	1 yr.	"
10	19	19.2	164	2 wks.	"	53	57	8.2	95	2 wks.	"
11	20	18.9	141	2 wks.	"	54	20	8.0	131	5 days	"
12	30	18.7	68	1 wk.	"	55	50	7.4	81	1 day	"
13	40	18.3	246	2 days	"	56	67	7.1	82	3 mos.	"
14	48	18.1	172	1 wk.	"	57	8	7.0	94	2 mos.	"
15	34	17.6	85	2 wks.	"	58	46	7.0	78	1 wk.	"
16	50	16.7	236	2 days	"	59	64	7.0	128	21 mos.	"
17	33	16.6	182	7 wks.	"	60	60	6.9	97	2 wks.	"
18	42	14.7	170	3 wks.	"	61	43	6.8	105	2 mos.	"
19	39	14.7	148	1 wk.	"	62	47	6.8	77	5 days	"
20	29	14.7	77	2 wks.	"	63	69	6.7	104	2 wks.	"
21	24	14.5	123	2 wks.	"	64	20	6.7	38	3 wks.	"
22	25	14.4	141	2 wks.	"	65	61	6.6	133	2 wks.	"
23	44	13.5	147	2 mos.	"	66	70	6.6	219	3 wks.	"
24	40	12.7	116	3 mos.	"	67	53	6.4	26	8 mos.	"
25	27	12.6	110	3 mos.	"	68	53	6.3	97	1 wk.	"
26	52	12.6	78	5 days	"	69	56	6.2	53	8 mos.	"
27	46	12.5	210	3 mos.	"	70	46	6.2	39	5 wks.	"
28	30	12.5	76	5 mos.	"	71	52	6.2	70	4 mos.	"
29	..	12.5	97	1 wk.	"	72	45	6.1	114	2 wks.	"
30	34	12.5	110	11 mos.	"	73	21	6.1	72	2 days	"
31	38	12.2	72	6 wks.	"	74	8	6.1	106	1 yr.	Reeovered
32	51	11.6	57	1 wk.	"	75	60	6.1	41	3 yrs.	Died
33	32	11.5	102	1 wk.	"	76	56	6.0	52	3 mos.	"
34	8	11.1	90	6 wks.	"	77	59	6.0	169	3 wks.	"
35	41	11.1	91	6 wks.	"	78	21	5.6	70	18 mos.	Recovered
36	36	11.1	139	1 wk.	"	79	50	5.5	62	3 mos.	Died
37	34	11.0	144	2 mos.	"	80	12	5.4	42	4 mos.	"
38	30	11.0	97	3 days	"	81	53	5.3	100	4 mos.	"
39	33	10.7	78	2 mos.	"	82	30	5.3	100	1 mo.	"
40	17	10.2	307	1 wk.	"	83	62	5.3	25	2 yrs.	"
41	26	10.0	112	3 wks.	"	84	29	5.2	65	1 wk.	"
42	30	9.8	62	7 mos.	"	85	21	5.1	42	5 mos	"
43	78	9.8	60	2 mos.	"						

*Abbreviated from Myers and Killian.[21] Cases 59, 75 and 83, marked unchanged in the original table, have since died.

Prognostic Value of the Blood Creatinine

In our studies on nitrogen retention[18, 19, 20, 21] it was soon noted that the creatinine of the blood was appreciably increased only after considerable retention of urea had already taken place and the nephritis was rather far advanced.* It was further observed that those cases in which the creatinine had risen above

*This point is well illustrated by the data in Table II of Chapter I, p. 19.

5 mg. per 100 c.c. of blood rarely showed any marked improvement, and almost invariably died within a comparatively limited time. The only exceptions were cases where the retention was due to some acute renal condition. In a recent paper[21] we have discussed in some detail the prognostic value of the blood creatinine in advanced nephritis and given in tabular form the results we have obtained since 1914. Data on 100 cases with high creatinines were presented, in 85 of which over 5 mg. of creatinine per 100 c.c. of blood were found. The data on these cases are given in abbreviated form in Table III, although for further details reference should be made to the above mentioned paper. As will be noted in the table the data are arranged in order of the magnitude of the blood creatinine, and include figures for the urea nitrogen on the same blood, the time under observation and the outcome of the case. These 85 cases included all the cases with over 5 mg. creatinine which had come under our observation since March, 1914 (to January, 1919), with the exception of three patients we had been unable to trace and who were presumably dead. Of these 85 cases, 83 are known to be dead, three having died (Cases 59, 75 and 83) since our last report. Of the two cases remaining alive, Cases 74 and 78 suffered from acute nephritis and have apparently recovered. Observations on Case 75 were made at frequent intervals during the past three years. This case remained nearly stationary for a longer period than any other case which we have encountered. Data on this case are given in Table IV. On his last admission to the

Table IV

Individual Observations on the Blood of Case 75

Date	Creatinine mg. to 100 c.c.	Urea N mg. to 100 c.c.	Uric acid mg. to 100 c.c.	CO_2 combining power c.c. to 100 c.c.
1917				
April 24	3.8	28	6.8	
May 8	6.1	41	5.5	50
15	5.2	30	7.7	
22	3.3	28	7.7	
June 1	3.1	36	10.4	
8	4.5	26	....	
1918				
April 2	3.8	57	....	
26	4.5	51	6.7	
1919				
Jan. 19	4.3	64		34
1920				
Feb. 18	7.2	56	5.0	

hospital the patient was definitely worse and the creatinine had risen to 7.2 mg. He died about four months after leaving the hospital. Even among the cases having very high blood creatinines there were many who were able to be up and about and some who showed considerable clinical improvement. It was in these cases that the blood creatinine gave a particularly good prognostic insight into the true nature of the condition. Case 25 is a good illustration of a patient in the last stages of the disease, but who, nevertheless, was able to be up and about (see Table V). Case 30 illus-

trates the value of the blood creatinine particularly well.* His blood showed a creatinine of 7.5 mg. when he first came under observation, and although he showed considerable clinical improvement over a period of nearly seven months, still his blood creatinine remained relatively constant, except for a temporary elevation to 12.5 mg. The dietetic measures which were employed to alleviate the nitrogen retention resulted in a gradual reduction of the urea nitrogen from 135 mg. per 100 c.c. to slightly below 30 mg. during this period. Despite his high creatinine (last determination 6.8 mg.), the patient left the hospital feeling well, returned to work as a guard on the subway and did not die until about five months later.

TABLE V

INDIVIDUAL OBSERVATIONS ON THE BLOOD OF CASE 25

DATE 1918	CREATININE MG. TO 100 C.C.	UREA N MG. TO 100 C.C.	URIC ACID MG. TO 100 C.C.	CO_2 COMBINING POWER C.C. TO 100 C.C.
March 12	8.6	97	8.1	31
19	9.4	102	7.0	..
22	12.6	110	...	17
26	12.1	110	...	28
29	11.9	...	...	28
April 16	11.2	78	...	23
30	13.3	81	...	24
May 7	15.2	76	...	25
24	17.0	148	...	12

This patient was discharged clinically improved on March 30, but came to the laboratory at our request for blood examination on April 16. At this time she apparently felt fairly well. She was readmitted to the hospital on April 27 and died on May 25.

It will be apparent from an inspection of Table III that there is no definite parallelism between the figures for urea and the creatinine. There are several cases with high creatinines in which the urea was not markedly elevated, notably Cases 64, 67, and 70, also Case 30 after prolonged dietetic treatment. Deductions based on the urea nitrogen alone in these cases would obviously have been quite misleading. In contrast to this, a good many cases have been encountered in which there was a marked retention of urea, although the creatinine was practically normal. In most of these cases improvement took place.

The highest blood creatinine we have observed with ultimate recovery is given in Table VI.[22] This patient developed anuria following a unilateral pyelogram and displacement of renal calculus. After the blood creatinine had risen to 23.1 mg. and the CO_2-combining power fallen to 21, alkali therapy was started and fluid forced with the aid of a duodenal tube, about 1000 to 4000 c.c. being given daily. The blood analyses in this case gave a clear understanding of the condition, without which the patient would unquestionably have died.

*Data on this case are given in tabular form in Chapter II (Table VI) on nonprotein and urea nitrogen, p. 37.

TABLE VI

CASE OF L. M., SHOWING MARKED NITROGEN RETENTION FOLLOWING NEPHROLITHIASIS WITH REFLEX ANURIA, RECOVERY*

DATE 1922	BLOOD ANALYSES					REMARKS
	UREA N	CREATININE	CHLORIDES AS NaCl	SUGAR	CO_2 COMBINING POWER	
	mg.	mg.	per cent	per cent	c.c.	
March 13	12.1		0.475	0.119		Uric acid 4.6 mg. on March 13.
March 27	62.5	12.5	0.413	0.230	50	Anuria March 18 to March 21 following pyelogram and displacement of renal calculus.
March 30	83.3	17.0	0.400	0.246	32	Phlebotomy March 31. Alkali therapy March 31—April 3.
March 31	92.0	23.1	0.357	0.290	21	
April 2	98.6	20.4	0.350	0.226	56	Forced fluid from March 31.
April 3	82.0	20.0	0.326	0.272	82	
April 4	104.0	21.2	0.300	0.332	63	
April 10	56.6	9.2	0.450		56	Passed calculus April 8.
April 15	48.0	4.4	0.538		63	
April 22	12.9	3.2	0.550	0.141	61	
May 4	9.7	2.7	0.513	0.109	56	Discharged May 15.
July 11	16.0		0.550	0.097		Uric acid 4.2 mg.

*Taken from McCarthy, Killian and Chace.[22]

That experimental nephritis in animals produces the same type of nitrogen retention found in human nephritis is nicely illustrated in Table VII. Rose and Dimmitt,[23] to whom the author is indebted for the table, have shown that mucic acid produces in rabbits a severe nephritis, the lesions

TABLE VII

NEPHROPATHIC ACTION OF MUCIC ACID

Rabbit No. 22, Male, 1970 gm.

DATE, 1922	WATER INTAKE	VOLUME OF URINE	PHTHALEIN	BLOOD ANALYSES					NOTES, ETC.
				NON-PROTEIN N	UREA N	CREATININE	SUGAR	NaCl	
March	c.c.	c.c.	per cent	mg.	mg.	mg.	per cent	per cent	
17	80	98	80	42.3	21.0	1.4	0.12	0.59	9 a. m.—6 c.c. blood
17									0.5 mucic acid 5 p. m. Acid given subcutaneously in 13 c.c., neutralized.
18	15	26	4	78.9	51.3	3.0	0.14	0.56	9 a. m.—8 c.c. blood
19	35	27							
20	55	54	2	177.6	154 0	8.2	0.20	0 56	9 a. m.—7 c.c. blood
21	45	87		202.7	161.5	8.5	0.20	0.53	9:15 a. m.—7 c.c. blood
22	80	74	3	223 8	190 0	8.5	0.23	0.52	9:10 a. m.—7 c.c. blood
23	100	96		240.0	211.2	8.5	0.27	0.52	9 a. m.—6 c.c. blood
24	105	104	12	243.5	214.0	8.3	0.35	0.53	9 a. m.—8 c.c. blood
25	105	114		214.0	180.7	7.4	0.33	0.54	9 a. m.—8 c.c. blood
26	100	115							
27				187.0	153.8	4.2	0.32	0.54	10 a. m.—7 c.c. blood Animal killed

Autopsy showed "Tubular nephritis with considerable evidence of secondary involvement of the glomeruli."

Unpublished observations of W. C. Rose, reproduced with the author's permission.

involving the tubules primarily, with more or less secondary involvement of the glomeruli. It will be noted that the blood creatinine rose in three days from 1.4 to 8.5 mg., but at the time the animal was killed ten days later it had fallen to 4.2 mg., a proportionally much greater drop than had taken place in the urea.

Our observations regarding the prognostic value of the blood creatinine appear to have received quite general confirmation, although only a few individuals have reported their findings. In 1916 Rosenberg[24] presented observations on the blood creatinine of quite a large series of nephritics which were in general confirmation of our findings, while the subject of the creatinine content of the blood has been discussed in considerable detail by Feigl.[25] Rabinowitch[26] has given a series of fourteen cases with blood creatinine figures above 5 mg., all dying in 1 to 120 days, while Rowntree[27] has recently reported observations on a comparatively large series of cases, which are in general confirmation of our findings. He states that the only patient to recover (with over 10 mg.) was one on whom a prostatectomy was performed. This patient was in fair condition, with fair renal function at the end of a year. We have observations on a somewhat similar case of a man, 55 years of age, who was brought into the hospital in a uremic condition with a creatinine of 11 mg. per 100 c.c. A cystotomy was immediately performed. Two months later at the time of the prostatectomy the creatinine had dropped to 4.6 mg. Thirteen months after admission the patient was feeling fairly well, although the creatinine was still 4.1 mg. His phthalein output was 5 per cent.

Theoretically, the amount of the increase of the creatinine of the blood should be a safer index of the decrease in the permeability of the kidney than the urea, for the reason that creatinine on a meat-free diet is entirely endogenous in origin and its formation (and elimination normally) very constant. Urea, on the other hand, is largely exogenous under normal conditions and its formation consequently subject to greater fluctuation. For this reason it must be evident that a lowered nitrogen intake may reduce the work of the kidney in eliminating urea, but can not affect the creatinine to any extent. Apparently the kidney is never able to overcome the handicap of a high creatinine accumulation. It would seem that creatinine being almost exclusively of endogenous origin, furnishes a most satisfactory criterion as to the deficiency in the excretory power of the kidneys and a most reliable means of following the terminal course of the disease, though it should be noted that urea, being largely of exogenous origin, is more readily influenced by dietary changes, and therefore constitutes a more sensitive index of the response to treatment.

Estimation of Creatinine

To Folin[15] is due the credit of describing in 1914 the first satisfactory method of estimating creatinine in blood, employing principles similar to his unique quantitative colorimetric method for creatinine in urine, introduced just ten years earlier. It should be noted, however, that in 1909 Shaffer and Reinoso[11] suggested a procedure for the estimation of creatinine in dilute solutions,

and with the aid of this method pointed out that normal dog serum contained about 1 mg. of creatinine per 100 c.c. Our first few determinations made on "uremic" blood were carried out following the suggestions of Shaffer and Reinoso. With the publication of Folin's paper we adopted his method for the most part, except that we still continued to carry out the preliminary dilution of the blood with water rather than saturated picric acid solution, for the reason that we regarded this the preferable way of analyzing the whole blood.[28] Later Hunter and Campbell made a very careful study of the various factors involved in the estimation of creatinine in blood. Although they originally made their creatinine observations on laked blood, following our suggestion,[29] they have obtained data[30] which tend to show that these results are somewhat too high (particularly in normal blood), owing to an interference in the color development on the part of some constituent in the corpuscles. Hunter and Campbell plotted time curves for the creatinine color reaction and found that the curve in the case of the plasma followed closely that of the pure creatinine solution, while when whole blood was employed, diluting with saturated picric acid solution according to Folin's technic, there was considerable deviation and that when the corpuscles were laked according to our technic, the deviation was even greater. For a ten minute interval of color development Hunter and Campbell obtained the following results: plasma, 1.06 mg. per 100 c.c.; whole blood not hemolyzed, 1.57 mg.; and whole blood hemolyzed, 1.84 mg. (1.72 mg. at the end of eight minutes). Similar observations regarding the increased color producing effect of hemolyzed blood were made a little earlier by Wilson and Plass.[31] Although, as just indicated, the accuracy of the creatinine estimation in normal blood is probably greater in plasma than in whole blood, the findings with normal whole blood are comparable, and the importance of the above source of error decreases with a rise in the creatinine content of the blood, so that the accuracy of the estimation in whole blood is probably greater with pathologic than normal values.

In order to overcome some of the difficulties incident to this determination, other protein precipitants have been tried aside from picric acid. Denis[32] has suggested the use of metaphosphoric acid and apparently obtained quite satisfactory results. In their recent system of blood analysis Folin and Wu[33] employ tungstic acid as their protein precipitant (see p. 151). Both of these methods appear to yield satisfactory results and provide also for a more accurate estimation of the creatine than is possible where picric acid is employed for the precipitation of the proteins. In a limited number of creatinine determinations which we have carried out with these methods the results did not materially differ from those obtained with the older method. Disadvantages of these methods are the added steps necessary, and especially the fact that they require a greater dilution of the blood (1 to 12½ and 15 instead of 1 to 5) resulting in a weaker color development. With normal bloods the color development is weak, and yellow colors at best are hard to match. Further dilution also increases the error incident to the sodium picrate. It will thus be seen that the disadvantages of these newer methods about balance their advantages.

In the case of bloods having a high creatinine content, however, the higher dilution called for in the Folin-Wu procedure is not only advantageous but necessary. With bloods high in creatinine a good rule to follow for any of the methods of precipitation is to so further dilute the blood that the filtrate contains close to 1 mg. creatinine per 100 c.c. of filtrate. This is necessary to effect a complete extraction of the creatinine.

One factor of very great importance in the creatinine estimation, no matter what technic is employed, is the picric acid. Before being used for this purpose all picric acid should be tested. Folin and Doisy[34] have suggested a very simple method: "To 20 c.c. of a saturated (1.2 per cent) solution of picric acid add 1 c.c. of 10 per cent sodium hydroxide and let it stand for 15 minutes. The color of the alkaline picrate solution thus obtained must not be more than twice as deep as the color of the saturated acid solution. * * * If the picric acid is unusually pure, the color of the picrate solution will not be more than one and a half times as deep as that of a saturated picric acid solution; *i.e.,* by setting the picric acid solution at 20 mm. in the Duboscq colorimeter, the picrate will give a reading of 13 to 14 mm." Benedict[35] has recently described a very simple and satisfactory method of purifying picric acid by means of recrystallization from benzene. As pointed out by Hunter and Campbell[29] saturated picric acid solutions develop a chromogenic substance on standing exposed to light, an observation we can verify. Therefore, any picric acid solutions used in this connection should be reasonably fresh.

In carrying out the picric acid precipitation Greenwald and McGuire[36] have followed our suggestion in laking the blood first, but regard shaking essential to render the solution saturated. We have always regarded preliminary stirring at intervals necessary to break up the lumps of blood and picric acid and make the mixture homogeneous. This seems to break up the lumps better than hand shaking alone. Behre and Benedict[16] also emphasize the necessity of shaking and advise placing the tubes in a shaking machine for 5 to 10 minutes after the preliminary stirring, if this method is to be followed.

It should be borne in mind that according to the observations of Behre and Benedict,[16] the chromogenic substance present in blood, which reacts with picric acid and alkali, is not creatinine. It is hard to believe, however, that this substance is not the precursor of the urinary creatinine.

For various reasons, given above, we have adhered essentially to our original method of estimating creatinine.

Method.—To 20 c.c. of distilled water in a 50 (or 25) c.c. centrifuge tube* are added 5 c.c. of the well-mixed oxalated blood (or oxalated plasma). This is then stirred with a glass rod until the blood is thoroughly hemolyzed, after which about 1 gram of dry picric acid (sufficient to completely precipitate the proteins and render the solution saturated) is added. The mixture is thoroughly stirred until it is uniformly yellow and then at intervals for twenty to thirty minutes, after which it is centrifuged and filtered. (As pointed out above

*Large centrifuges which will take 50 and 100 c.c. tubes are unavailable in many laboratories, and the ordinary 15 c.c. conical centrifuge tube can not satisfactorily be used for this purpose, as they are very difficult to clean. In order to adapt the small electric centrifuge to this work, the Sorensen Company, New York City, have prepared a special trunnion cup for their small centrifuge which takes cylindrical centrifuge tubes of 25 c.c. capacity. (See Fig. 3, p. 77.) Although these tubes hold slightly more than 25 c.c., it is best to employ 4 c.c. of blood and 16 c.c. of water in making the determination. For the cup of the Bock-Benedict colorimeter not more than 5 c.c. of filtrate are needed, in which case the color may be developed on this amount of filtrate with 0.25 c.c. of 10 per cent sodium hydroxide, if desired.

there are advantages in stoppering these tubes and placing them in a shaking machine, after the preliminary stirring.) Sufficient filtrate is obtained for the estimation of both the creatinine and the sugar (also the blood chlorides where 5 c.c. of filtrate are used for the creatinine). To 10 c.c. of this filtrate is added 0.5 c.c. of 10 per cent sodium hydroxide and a similar amount of alkali added to each of three standards (10 c.c. of standard creatinine* in saturated picric acid, containing 0.3, 0.5 and 1.0 mg. creatinine to 100 c.c. of picric acid). A standard is selected which approximates the color intensity of the unknown, and set at the 15 mm. mark. With bloods showing much over 5 mg. creatinine it is desirable to make a 1 to 10 or 15 dilution of the blood so that when the color is developed on the filtrate it will closely match the standard (1.0 mg.), i. e., read between 12 and 18 mm. with the standard at 15 mm. (In an emergency where sufficient blood is not available for a second dilution, it may be allowable to secure the added dilu-

Fig. 3.—Small centrifuge adapted to chemical blood work.

tion by further diluting the 1 to 5 picric acid filtrate. However, with blood creatinine figures of over 5 mg. the 1 to 5 dilution is not entirely adequate for a complete extraction of the creatinine.) The colors are compared after they have been allowed to develop for eight minutes.

Calculation.—For the calculation the following formula may be used: $\frac{S}{R} \times S_1 \times D =$ mg. creatinine to 100 c.c. of blood, in which "S" represents the depth of the standard (15 mm.), "R" the reading of the unknown, "S_1" the strength of the standard and "D" the dilution of the blood. For example, with a reading of 15, a standard of 0.3 mg. and a blood dilution of 5 the formula would work out: $\frac{15}{15} \times 0.3 \times 5 = 1.5$ mg. creatinine to 100 c.c.

*Pure creatinine may be prepared by the admirable method of Benedict.[37] A standard solution of creatinine, 1 mg. to 1 c.c. is kept in 0.1 N hydrochloric acid, and from this the various solutions are prepared with saturated picric acid solution, 100 or 200 c.c. at a time.

Estimation of Creatinine With the Test Tube Colorimeter

Method.—The technic is as follows: 3 c.c. of the well-mixed blood are treated with 12 c.c. of water (4 volumes) in a 15 to 20 c.c. cylindrical centrifuge tube (test tube). After the corpuscles have been laked, about 0.5 gm. of dry picric acid is added, and the mixture stirred at intervals with a glass rod until it is a light yellow. When the protein precipitation is complete, the tube is centrifuged and the supernatant fluid filtered through a small filter paper. As noted above it is important that this filtrate should be thoroughly saturated with picric acid. (Sufficient material is also available for the sugar estimation; see next chapter.) To 5 c.c. of the filtrate in a test tube is added 0.25 c.c. of 10 per cent sodium hydroxide and a similar amount of alkali added to 3 standards in test tubes of the same caliber (5 c.c. of each of 0.3, 0.5 and 1.0 mg. creatinine to 100 c.c. of picric acid). At the same time 1.0 c.c. of the sodium hydroxide is added to 20 c.c. of saturated picric acid solution to make a diluting fluid. After 8 minutes have elapsed a standard is selected which is slightly lighter than the unknown. This is poured in the left-hand tube and the unknown into the right-hand tube (the tubes should be perfectly dry). The test tube which held the unknown is now rinsed out with the diluting fluid and the unknown diluted to correspond with the standard.

Calculation.—To calculate the mg. of creatinine per 100 c.c. of blood, the following formula may be used, in which "S" represents the strength of the standard (0.3, 0.5 or 1.0 mg. to 100 c.c.), "D" the dilution in c.c. of the unknown required to match the standard, and "B" the amount of blood (1 c.c.) employed in c.c.: $\frac{S \times D}{B}$ less 5 per cent to allow for addition of alkali = mg. creatinine per 100 c.c. of blood, or briefly, since the equivalent of 1 c.c. of blood is employed, multiply the strength of standard by the dilution in the unknown tube and deduct 5 per cent to allow for the addition of the alkali. For example, if the 0.5 standard is used, and the dilution of the unknown is 7.2 c.c., the result would be 3.6 mg. to 100, less 5 per cent, i.e., 3.4 mg.

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CHAPTER V

BLOOD SUGAR

THAT blood may contain a sugar-like substance was first recognized in 1775 by Dobson, although it was not until seventy years later that its presence in normal blood was discovered by the noted French physiologist, Claude Bernard, who made many of our classic observations on carbohydrate metabolism. By means of his sugar *piqûre* he first noted the connection between hyperglycemia and glycosuria (glycuresis). It remained for Lewis and Benedict[1] in 1913 to introduce a colorimetric method for blood sugar estimation so simple that it could readily be employed for clinical as well as scientific purposes. Earlier in the same year Bang[2] had described a very ingenious method requiring only two to three drops of blood, but the fact that it was a gravimetric-volumetric procedure precluded any very extensive clinical application.

Stimulated by these methods many studies dealing with the sugar of the blood appeared during the succeeding five years, while previous to this time Bang[3] had written a very interesting monograph under the title "Der Blutzucker." Obviously reference can be made here to only a few of these papers. Among these might be mentioned the papers of Hopkins,[4] Geyelin,[5] Hamman and Hirschman,[6] Denis, Aub and Minot,[7] Mosenthal, Clausen and Hiller,[8] Janney and Isaacson,[9] Bailey,[10] Williams and Humphreys[11] and Allen, Stillman and Fitz.[12]

If we may rely upon the findings with the Benedict method, the blood sugar of the normal human subject falls somewhere between 0.09 and 0.12 per cent, on the average being 0.10 per cent. There are a considerable number of miscellaneous hospital cases, however, which show blood sugars of 0.12 to 0.14 per cent. These figures represent observations made in the morning previous to the intake of any carbohydrate. After a meal rich in carbohydrate there may be an appreciable rise in the sugar content of the blood, while after the intake of even moderately large amounts of glucose, the hyperglycemia may be sufficient to induce a slight temporary glycosuria (glycuresis).*

Conditions of hyperglycemia are much more common and of greater clinical interest than those of hypoglycemia, owing primarily to the fact that diabetes belongs to the former group. Among other conditions which frequently show a moderate hyperglycemia are nephritis and hyperthyroidism. Hyperglycemias are also found in pancreatic disease, in some cases of cholecystitis and in some infections. Hypoendocrine function (pancreas excepted) would appear to result in hypoglycemia, and comparatively low blood sugars have

*As it is now generally recognized that sugar is present in normal urine (to the extent of about 0.05 to 0.20 per cent), the term glycosuria is somewhat of a misnomer. It implies no more than glycemia does in the case of blood. We might speak of a hyperglycosuria in the same way that we refer to a hyperglycemia, or better employ the term "glycuresis" suggested by Benedict.[13] This would indicate an increase in the sugar content of the urine to the extent that it could be detected by *ordinary* qualitative tests, and not a new appearance of sugar.

been observed in myxedema, cretinism, Addison's disease, pituitary disease and other less clearly defined endocrine disorders such as muscular dystrophy.

Relation of Glycosuria to Hyperglycemia and the Threshold Point

All forms of glycosuria are accompanied by hyperglycemia, if we except the glycosuria produced by such substances as phlorhizin and uranium, and the analogous clinical condition, "renal diabetes." In mild cases of diabetes the hyperglycemia is not excessive, generally 0.2 to 0.3 per cent, although in severe cases figures up to and even above 1.0 per cent have been obtained. The normal threshold of sugar excretion (i.e., the point of glycuresis) is about 0.16 to 0.18 per cent. With blood sugar concentrations of 0.15 to 0.20 per cent the appearance of sugar in the urine is apparently dependent on whether or not diurèsis exists, glycosuria appearing especially in the latter case. When the threshold point has been passed, however, the overflow of sugar into the urine may continue until the concentration in the blood has fallen nearly to normal. Mild cases of diabetes usually have a normal threshold, although some severe cases apparently have a lowered threshold, increasing the severity of the condition. Ordinarily in the early stages of the disease there is a fairly direct relationship between the hyperglycemia and glycosuria. In the later stages of the disease, however, cases are frequently encountered with marked hyperglycemia and only slight glycosuria, showing that the threshold point has been raised. The cause of glycosuria in "renal diabetes" is obviously due to the reverse condition, viz., a threshold point below the level of the normal blood sugar.

Diabetes Mellitus

Ever since the discovery by von Mering and Minkowski in 1889 that pancreatectomy greatly lowered the carbohydrate tolerance and produced a pronounced glycosuria, the pancreas has been looked upon by most workers in this field as the crux of the diabetic problem. The pathological studies of Opie published in 1900 focused the attention of investigators upon the rôle of the islands of Langerhans, but the definite experimental proof of the so-called "island theory" was very illusive. Many physiological researches were carried out in an effort to demonstrate a relation between the pancreas and carbohydrate metabolism, some of which, as we now see, fell just short of being successful. It remained for a young investigator with the courage of his convictions to actually demonstrate this internal secretion. The isolation of the pancreatic hormone, insulin,* in highly potent form was first accomplished in 1921 by F. G. Banting[14] in collaboration with C. H. Best, while working in Macleod's laboratory at Toronto. Banting's hypothesis, which covered the failure of previous workers with pancreatic extracts, was that trypsinogen or its derivatives were antagonistic to the internal secretion of the gland. He first conceived the idea of preparing an effective extract from

*During proof reading an important issue of the *Journal of Metabolic Research* appeared which is entirely devoted to various factors related to the clinical use of insulin. This issue was sent to press in May 1923, although nominally Nos. 5 and 6, Nov.—Dec., 1922, ii, 547-985. It contains papers by Banting, Campbell and Fletcher, Joslin, Wilder and Boothby, Williams, Fitz, Geyelin and Harrop, Woodyatt, Allen and Sherrill.

the atrophied pancreas after ligation of the ducts, following which there is degeneration of the acinous, but not of the islet tissue. The extract of the atrophied pancreas was found to be nontoxic and highly potent in the treatment of depancreatized dogs. Without further reference to many fundamental observations, suffice it to say that with the actual demonstration of the presence of this internal secretion in the pancreas, efforts were made to secure easier methods of preparing a potent but nontoxic extract. This was ultimately accomplished by extracting hashed adult beef pancreas with 95 per cent alcohol containing a small percentage of hydrochloric acid, after which the material was further concentrated and ultimately precipitated by strong alcohol. The potent material employed is essentially an aqueous solution of this precipitate.

It is not within the scope of the present book to enter into a discussion of the clinical use of insulin, except insofar as a knowledge of chemical blood findings is necessary. It might be said, however, that data on the blood sugar and in certain cases of the CO_2 combining power are absolutely essential to the intelligent use of insulin.

The unit of insulin originally adopted was the amount required to lower the normal blood sugar of a one kilogram rabbit to 0.045 per cent, at which point convulsions generally occur. This amount of insulin, when injected into a human diabetic will enable the patient to utilize or store from 1 to 4 (average 2.5) grams additional carbohydrate depending upon the severity of the condition. The amount of insulin administered is generally gauged by the height of the hyperglycemia. In case of an overdose, resulting in a marked hypoglycemia, the unfavorable symptoms, hunger, weakness, nervousness and sweating, may be counteracted by giving sugar by mouth, or if necessary, glucose intravenously. Subcutaneous injections of adrenaline may also be employed, the object in either case being to restore the blood sugar to normal. Insulin is generally best administered (subcutaneously) 15 to 30 minutes before a meal, and once, twice or three times a day (more in the morning than in the evening) according to the severity of the diabetes. It apparently enables a diabetic to utilize carbohydrates in a normal manner.

TABLE I

SOME BLOOD AND URINE FINDINGS AFTER INSULIN, ON A. Y., AGED 20 YEARS
21 units of "Iletin" given at 10:10 A. M.

TIME, JAN. 10, 1923	BLOOD ANALYSES			TIME	URINE ANALYSES			
	SUGAR	DIASTATIC ACTIVITY	CO_2 COMBINING POWER		VOLUME	SUGAR	SUGAR	ACETONE BODIES
	per cent		c.c. to 100		c.c.	per cent	gm.	gm.
10:00	0.302	24	37	10:20	210	3.5	7.35	0.76
11:10	0.274	24	40	11:20	65	3.0	1.95	0.29
12:10	0.220	17	53	12:20	30	1.5	0.45	0.10
				1:20	305	0.01	0.03	0.06
2:10	0.184	13	57	2:20	255	0.01	0.02	0.03
3:15	0.193	15	52	3:20	45	0.11	0.04	0.08
4:15	0.157	18	51	4:20	115	0.08	0.09	0.06
5:10	0.190	18	51	5:20	35	0.19	0.09	0.10

The observations recorded in Table I, taken from a series of unpublished analyses made by Dr. J. A. Killian in the author's laboratory on patients of Dr. H. O. Mosenthal, give an excellent idea of the action of insulin in diabetes. As will be noted the influence upon the urine sugar is more rapid and much more striking than in the case of the blood sugar. Obviously the sugar threshold is an important factor in the disappearance of sugar from the urine, the threshold in this case being close to 0.2 per cent. Whereas the lowest urine sugar was reached at the end of three to four hours, the lowest blood sugar, 0.157 per cent, was not obtained until the sixth hour. After the blood sugar has dropped below the threshold point, and as noted below, this is often raised in diabetes, the urine furnished no further information. Chiefly for this reason the blood sugar is a much more reliable guide for the insulin treatment, despite the striking influence upon the urine sugar; and it is better that at the lowest point there should still be a slight hyperglycemia, as in this case, than that the dangers of a marked hypoglycemia should be encountered.

Probably the most valuable action of the insulin in this case, however, was the influence on the alkaline reserve, as shown by the CO_2-combining power. This rose 20 volume per cent in four hours, a rise which would have otherwise required the administration of 30 grams of sodium bicarbonate. It will be noted that the highest alkaline reserve was found at a time when the elimination of acetone bodies in the urine had dropped to a minimum of 30 mg. per hour. In harmony with the findings on this case Banting, Campbell and Fletcher[15] have already stated that insulin may be considered a specific in the treatment of diabetic coma.

It has long been recognized that oxidation of carbohydrate was necessary to the oxidation of fat, and the expression of Rosenfeld that fat burns in the flame of carbohydrate is often cited. Through the recent work of Shaffer on antiketogenesis it has been shown that under the most favorable conditions one molecule of glucose can take care of two molecules of fatty acid, but that any fat in excess of this is incompletely oxidized and gives rise to the formation of the ketone bodies. Since insulin enables the organism to utilize carbohydrate, it indirectly enables it to metabolize fat. Its action in diabetic coma is therefore readily apparent.

Just how insulin exercises its action is not entirely clear. It has been generally recognized that there are two processes at work in diabetes, one an increased mobilization of sugar, the other a decreased oxidation. That insulin influences both of these processes seems unquestioned. The severe diabetic has very little power of storing glycogen, but insulin appears to temporarily restore this, possibly by a restraining influence upon the glycogenolytic (diastatic) ferment. How it aids in the oxidation of sugar is not evident, although Winter and Smith[16] have recently pointed out that insulin lowers the rotary power of glucose, and have suggested that insulin may activate the enzyme responsible for the conversion of α-β glucose to γ glucose, in which form it is supposed that glucose is oxidized.

The Sugar Threshold in Diabetes

Since the glycosuria of diabetes mellitus is dependent upon hyperglycemia, it is apparent that this latter condition is more fundamental than the glycosuria. Glycosuria is evidently only a safety factor, and the internist in applying his dietetic or other treatment should endeavor primarily to relieve the hyperglycemia; although in so doing he relieves the glycosuria, he should be finally guided by the concentration of sugar in the blood. In the early stages of the disease the glycosuria is an excellent index of the hyperglycemia, but when the threshold point has been raised, as for example in diabetes associated with chronic kidney disease, the disappearance of sugar in the urine is a rather poor guide to the glycemia. As will be observed in Table II,

TABLE II

INFLUENCE OF NEPHRITIS UPON THE EXCRETION OF SUGAR IN DIABETES

CASE	AGE	SEX	SUGAR OF BLOOD PER CENT	SUGAR OF URINE PER CENT	SEVERITY OF NEPHRITIS	CASE	AGE	SEX	SUGAR OF BLOOD PER CENT	SUGAR OF URINE PER CENT	SEVERITY OF NEPHRITIS
1	63	♀	0.19	0	++	11	53	♂	0.37	1.7	++
2	47	♂	0.22	0	++	12	35	♀	0.38	6.2	?
3	53	♂	0.24	1.7	+	13	48	♀	0.39	2.2	++
4	57	♀	0.24	5.0	++	14	30	♂	0.42	5.0	?
5	27	♀	0.31	6.0	+	15	46	♀	0.42	3.6	+++
6	61	♀	0.33	0.5	++	16	50	♀	0.46	0	+++
7	61	♂	0.34	6.3	+	17	56	♀	0.57	8.0	+
8	68	♂	0.35	0	+++	18	15	♂	0.79	8.7	+
9	23	♀	0.36	7.0	—	19	53	♂	0.98	1.6	++
10	47	♂	0.36	1.2	+++	20	52	♀	1.10	0.5	++

Data obtained previous to beginning treatment; taken from Myers and Bailey.[17]

blood sugar figures of 0.2 to 0.3 per cent, and even more, may be noted without the appearance of any sugar in the urine. Although some cases with definite nephritic symptoms retain the power of secreting a urine of high sugar content, severe nephritis appears to reduce markedly the permeability of the kidney for sugar; and it is only when these latter cases are excluded from the above table that there appears to be any relation between the hyperglycemia and glycosuria. Although this table probably gives an exaggerated impression of the occurrence of nephritis in diabetes, since the cases were those needing hospital care, it is not believed that the importance of this subject has been fully appreciated, although previously discussed by the older workers.[3, 18] Fitz[19] has presented evidence to show that impaired renal function exists in many cases of advanced diabetes. Williams and Humphreys[11] have made a study of the blood sugar threshold in diabetes and have observed in general that as age advances, the threshold rises. In connection with treatment they state: "Our experience has lead us to the conclusion that it is desirable to maintain the blood sugar level as nearly normal as possible even though severe restrictions in diet may be necessary for the purpose, notwithstanding the fact that in most cases the high threshold will permit of a much more liberal diet without the appearance of sugar in the urine." In discussing the high blood sugars which are observed in some diabetics on a protein-fat diet, Mosenthal[8] has

suggested that this may be merely a protective measure to adjust carbohydrate metabolism for the more advantageous utilization of glucose.

It is worthy of note in this connection that several interesting contributions dealing with the action of certain salts on the permeability of the kidney for sugar and the sugar threshold have come from Hamburger's[20] laboratory.

Diastatic Activity and Hyperglycemia

In 1917 Myers and Killian[21] described a simple method of estimating the diastatic activity of the blood and called attention to the fact that conditions of hyperglycemia were associated with an increased diastatic activity, and suggested that this might be an important factor in the production of the hyperglycemia in both diabetes and nephritis. The increase in the diastase of the blood in nephritis finds probable explanation in the decreased excretion of diastase in the urine, now well known in this condition, although a satisfactory explanation of the increased activity in diabetes is not so readily given. De Niord and Schreiner[22] have noted the exception that diabetics who are also syphilitic do not show a high diastatic activity. The liver has long been known to possess an active glycogenolytic ferment, and it seems logical that it should be the chief source of the blood diastase. This ferment cannot come from the pancreas since total pancreatectomy increases the diastatic activity of the blood,[21] while a liver poison like hydrazine lowers the activity.[23] The pancreas apparently exerts a restraining influence upon the glycogenolytic ferment of the liver (and blood), probably through its internal secretion, insulin. Normally the balance between the diastatic ferment and the internal secretion of the pancreas appears to be maintained in such a way as to keep the blood sugar within well defined limits.

The influence of insulin on the diastatic activity is shown in Table I, while Table III gives values for blood sugar and diastatic activity in normal individuals, diabetics, nephritics, illustrative endocrine cases, carcinoma of the pancreas, pancreatitis and cholecystitis. It will be noted that with the three normals the diastase was 16 and 17 and the blood sugar 0.1 per cent. Cases 4 and 5 are especially interesting, both being physicians whose blood diastase was estimated incidentally to other tests. High diastatic activities being observed, a history of so called "alimentary" glycosuria was elicited in each case. Of the diabetics, Case 7 was on a rigidly restricted diet throughout the period of observation. This serves to explain the comparatively low figures for blood sugar with the high diastase figures, while, on the other hand, the high diastatic activity affords an explanation as to why the restriction in the diet was unable to bring the blood sugar down to normal The improvement in Case 8 was very rapid as is evident by the marked drop which occurred in both the diastatic activity and the sugar in six days. The parallel fluctuations in the diastase and urea in Case 9 would suggest that the increased diastatic activity in nephritis is a retention phenomenon. Hyperfunction on the part of the ductless glands appears to result in an increase in the blood diastase and hypofunction (pancreas excepted) in the reverse effect, as shown by the data on Cases 11 and 12. In carcinoma of the pancreas, pancreatitis and in some

TABLE III

DIASTATIC ACTIVITY OF THE BLOOD IN NORMAL CASES, DIABETES, NEPHRITIS, ENDOCRINE CONDITIONS AND CARCINOMA OF THE PANCREAS

CASE	AGE	SEX	DIASTATIC ACTIVITY	SUGAR	UREA N	CO_2 COMBINING POWER	REMARKS
				PER CENT	MG. TO 100	C.C. TO 100	
1. J. K.	25	♂	17	0.10	15		
2. A. L.	27	♂	17	0.10	14		Normals
3. R. H.	28	♂	16	0.10	14		
4. E. H.	36	♂	34	0.16	13		Glycosuria noted on one occasion, slight proteinuria.
			29	0.11	12		
5. Z. D.	53	♂	29	0.12	13		Glycosuria noted on two occasions.
6. F. P.	51	♂	74	0.55		54	
			47	0.28			
7. F. M.	26	♂	59	0.17		49	Diabetes, Case 7 being on a rigidly restricted diet during this period.
			44	0.16		56	
			43	0.17			
			39	0.18			
			41	0.20			
8. L. M.	62	♀	42	0.26			
			24	0.12			
9. J. B.	34	♂	32	0.14	60		
			32	0.14	62	32	
			41	0.16	135		
			38	0.16	110		Nephritis
10. J. M.	41	♂	30	0.16	47	47	
11. C. G.	24	♀	24	0.13			Hyperthyroidism
12. J. S.	24	♂	10	0.09	13		Addison's disease
13. T. L.	43	♀	45	0.15			Carcinoma of the pancreas
14. D. M.	63	♂	45	0.14			
15. C. C.	50	♀	38	0.17			Pancreatitis, operation.
16. B. W.	38	♀	31	0.15			Pancreatitis, operation.
17. E. F.	56	♀	19	0.24	13	60	Cholecystitis.
18. A.McC.	46	♂	26	0.18	15	65	Cholecystitis, interstitial hepatitis.

cases of cholecystitis there is an increase in the diastatic activity of the blood as shown by Cases 14 to 18.

Renal Diabetes

So-called "renal diabetes" has been the subject of considerable discussion since Lépine in 1895 postulated the existence of this rather interesting condition in which the glycosuria is actually the result of renal disease, and not due to hyperglycemia as in diabetes. Some thirty cases had been recorded in the literature up to 1920, although the condition is probably not as uncommon as this number might imply. A few of these cases have shown definite evidence of renal disease aside from the glycosuria, but some would appear to be entirely free from the symptoms ordinarily associated with disease of the kidney. Renal diabetes has often been compared with phlorhizin glycosuria, demonstrated by von Mering in 1886, in which condition we have glycosuria without hyperglycemia. Some of the cases, however, appear to find a more direct analogy in the glycosuria of uranium nephritis, in which there is only a mild glycosuria, with a normal or nearly normal glycemia and a constant proteinuria. Data on four cases which might be put in the category of renal

TABLE IV

OBSERVATIONS ON RENAL DIABETES

CASE	AGE	SEX	DATE	SUGAR OF BLOOD	SUGAR OF URINE	REMARKS
				PER CENT	PER CENT	
1. M. B.	31	♀	8/25/15	0.09	1.3	Renal diabetes, diet low in carbohydrate on first test, regular diet on second test.
			10/13/16	0.11		
					1.2	
2. P. L.	10	♀	5/12/16	0.12	0.4	Cases of parenchymatous nephritis with fairly constant mild glycosuria.
			8/ 7/16	0.14	0.6	
3. J. P.	62	♂	8/27/15	0.18	1.1	
			5/29/16	0.15	1.1	
4. C. M.	54	♂	4/27/15	0.19	0.3	
			12/ 7/15	0.17	4.5	

diabetes are given in Table IV. The first of these was a very typical case of renal diabetes. Bailey[24] has already presented a report of this case (1) and also Case 3 with a general discussion of this subject. The slight glycosuria in the last three cases was associated with parenchymatous nephritis and was quite independent of the mild hyperglycemia found in the last two cases. From the above discussion it is apparent that a satisfactory diagnosis of renal diabetes cannot be made without a knowledge of the blood sugar. Now that this determination may be so easily made it seems probable that many cases presenting a history of a low grade glycosuria but without the classic symptoms of diabetes mellitus, will be definitely recognized as cases of renal diabetes.

Nephritis

It has been recognized for some time that many cases of severe nephritis show high figures for blood sugar. We have presented several papers giving such data,[17, 21] while Williams and Humphreys have made this subject the topic of a special paper.[11] They found that the range of the blood sugar levels was from 0.08 to 0.25 per cent, varying directly with the severity of the disease.

TABLE V

BLOOD SUGAR OBSERVATIONS IN SEVERE NEPHRITIS

CASE	AGE	SEX	DATE	SUGAR	CREATININE	UREA N
				PER CENT	MG. TO 100 C.C.	MG. TO 100 C.C.
1. E. M.	39	♂	12/ 4/15	0.225	21.5	129
			12/ 7/15	0.180	21.8	129
2. J. B.	34	♂	1/26/17	0.195	8.2	53
			2/23/17	0.114	7.9	61
			2/27/17	0.120	7.5	45
			3/ 2/17	0.117	6.6	44
3. F. F.	8	♂	12/18/17	0.106	6.1	106
			12/21/17	0.150	5.9	93
4. A. N.	27	♀	3/11/18	0.181	8.6	97
			3/19/18	0.131	9.4	102
			3/22/18	0.120	12.6	110
			3/26/18	0.161	12.1	110
5. M. D.	30	♀	5/27/20	0.121	6.5	42
			6/ 1/20	0.121	5.5	43
6. F. M.	57	♀	6/ 3/20	0.153	4.8	59
			6/18/20	0.096	3.5	43
7. A. D.	66	♀	6/ 5/20	0.163	2.8	30
8. M. I.	75	♀	6/16/20	0.172	7.0	121
			6/21/20	0.190	5.7	147

Hamman and Hirschman[6] have studied the sugar tolerance in nephritis and state that in many cases there is a profound change in carbohydrate metabolism, the blood sugar curve after the ingestion of glucose resembling the "diabetic curve." When there is marked interference with renal function very small amounts of sugar or none appear in the urine, although the blood sugar may go above 0.2 per cent. Bailey[10] has presented data on a very interesting case of nephritis where the blood sugar rose to 0.3 per cent before a slight glycosuria occurred. No satisfactory explanation of the hyperglycemia has been offered, although the suggestion has been made that some disturbance in the adrenals or other endocrine glands may be responsible for both the high blood pressure and the increased blood sugar. As already noted Myers and Killian[21] have observed an increased diastatic activity of the blood in this condition. Hyperglycemia data in nephritis are given in Tables III and V. It should perhaps be noted here that Morgulis and Jahr[25] have criticized the findings of high blood sugars in nephritis on the basis of error due to interference of creatinine in the sugar estimation. From an inspection of Table V, however, it is apparent that there is little relation between the height of the blood creatinine and the sugar. This criticism has been further discussed in connection with the method below. While there is probably some increase in other reducing substances in nephritic bloods, the evidence supports the view that this is largely due to sugar.

Endocrine Conditions

That cases of hyperthyroidism frequently show high blood sugars (although not often in the fasting state), and that alimentary glycosuria may be readily provoked in this condition has been recognized for some time. Blood sugar observations obtained after glucose tolerance tests have given us much more definite information regarding the carbohydrate tolerance of individuals with endocrine disorders than the older studies made on the urine. Experimental proof that hypoglycemia results from hypo-endocrine function was obtained by Janney and Isaacson[9] in the case of the thyroid, where hypoglycemia regularly developed after thyroidectomy. Low blood sugar values, figures ranging from 0.06 to 0.09 per cent, have been reported in myxedema,[5] cretinism,[9] Addison's disease, pituitary disease and other less clearly defined conditions such as muscular dystrophy. The last mentioned condition has been discussed by Janney, Goodhart and Isaacson[26] and McCrudden and Sargent.[27] Altogether twelve cases were reported, the blood sugars ranging from 0.064 to 0.086.

Carbohydrate Tolerance Tests

The advent of a simple method for blood sugar has made possible the estimation of the blood sugar concentration at short intervals after the administration of carbohydrate, usually glucose. With this method of study it has been possible to obtain much more consistent data regarding the carbohydrate tolerance than with similar tests formerly carried out on the urine, chiefly for the reason that the threshold point of renal excretion is not here a factor in the test. This test has served to put many of our older views, especially those regarding glucose

tolerance in endocrine disorders, on a more firm foundation. A number of the papers to which reference has already been made present data obtained with this method of study. The papers of Hamman and Hirschman,[6] and Williams and Humphreys[11] nicely demonstrate the great value of this method particularly in cases of diabetes and nephritis. Bailey[10] has presented quite extensive data on a few cases covering a wide variety of conditions and emphasizes the value of obtaining blood specimens at quarter hour intervals during the first hour and at half hour intervals thereafter until the sugar concentration returns to normal. With this technic the character of the curve is very clearly brought out. In practical work, however, this large number of determinations is not always possible. As a rule four sugar estimations at one hour intervals will serve to show the type of curve, despite the fact that the highest point in the curve may frequently be missed.

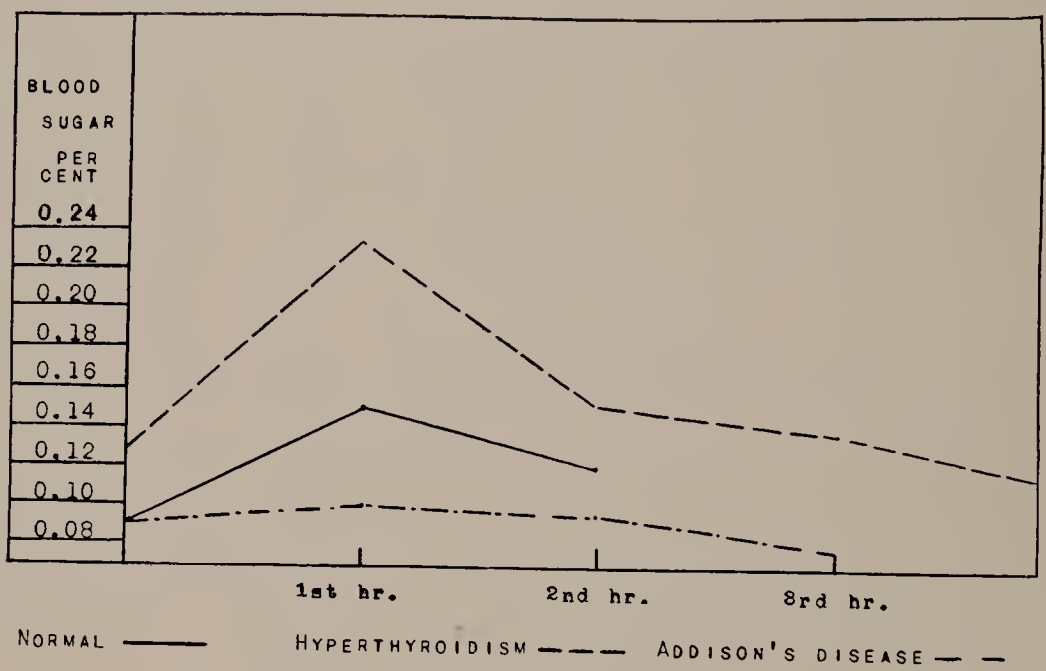

Fig. 4.—Illustrative glucose tolerance tests.

The method employed in this laboratory by Killian[28] is as follows: The patient is given, the first thing in the morning, a standard breakfast consisting of two slices of bread, one egg in any form and one cup of water. Two hours after this breakfast the patient empties the bladder and then receives 200 c.c. of water to drink. One hour after this a specimen of blood and a specimen of urine are taken to serve as controls. The patient then takes glucose, 1.75 grams per kilo of body weight in 50 per cent solution. Specimens of blood are now taken every hour for three to four hours, the sugar determined as described below and a curve plotted. Following the taking of glucose a 24-hour specimen of urine is collected and the glucose determined with the Benedict methods, by titration if the amount is large, otherwise by the colorimetric method.[29] (For methods, see p. 192.)

Many tolerance tests have been carried out on the fasting condition, but there are certain advantages in the use of the preliminary breakfast given above. It is well known that the tolerance for glucose is lower in the fasting condition than after glucose has been given, apparently as a result of its stimulus to the glycogen forming mechanism (due possibly to the liberation

of insulin by the pancreas). At the end of three hours this initial stimulus still appears to be sufficient to enable the normal organism to readily handle the amount of glucose given without undue hyperglycemia, i.e., above 0.15 per cent.

It is not possible in the compass of the present chapter to describe in detail the characteristics of the different types of curves obtained, although an idea of the curves observed in hyper-and hypo-endocrine conditions may be obtained from Fig. 4. This chart presents unpublished observations of Killian, showing a severe case of hyperthyroidism and a case of Addison's disease contrasted with a normal.

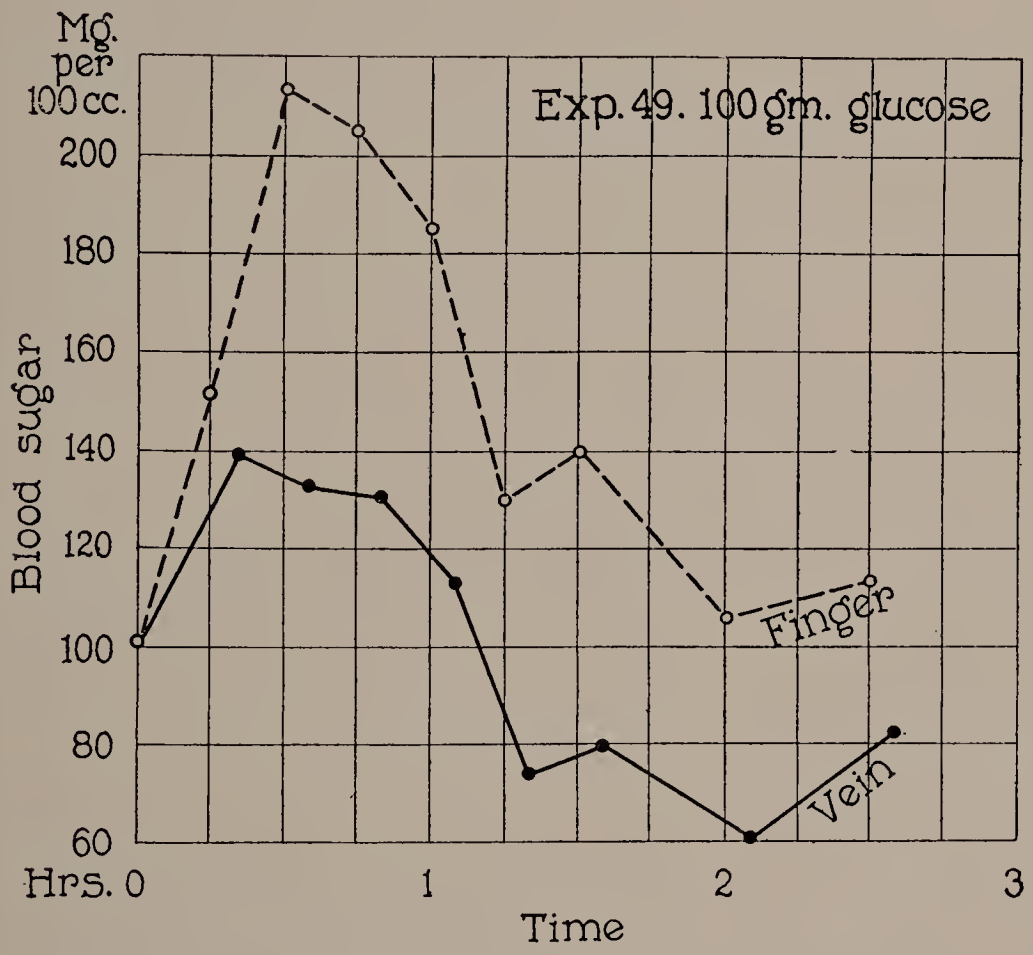

Fig. 5.—Glucose tolerance as shown by both finger and venous blood (Foster).

Practically all of the observations to which reference is made here have been carried out on blood obtained by venous puncture. In the light of the results obtained by Foster[30], discussed below, it would appear that much of the work carried out on pathological conditions might be repeated to advantage with this method, since it appears to furnish important information relative to glycogen formation.

In the past no distinction has been made between carbohydrate tolerance tests conducted on venous blood or finger blood. Foster[30] has recently pointed out that blood obtained by finger puncture is virtually arterial blood. He has found that although the sugar concentration of venous and finger (also

arterial) blood is the same in the fasting state, after the ingestion of glucose there is a more rapid and greater rise in the finger blood than in venous blood. This is beautifully illustrated in Fig. 5. taken from Foster. It will be noted that whereas the highest point observed for the venous blood sugar after the ingestion of 100 grams of glucose by a normal subject was 0.140 per cent, 0.214 per cent was reached in the finger blood. The logical explanation for this would appear to be found in glycogen formation in the muscle tissue, since the muscle stands between the arterial and venous blood. It is also apparent that when this glycogen synthesis has once been started it carries the sugar of the blood below the initial level as a result of "overactivity" of the glycogen forming mechanism. Additional doses of glucose given at this time have little hyperglycemic effect. Frutose and galactose, in harmony with the fact that they are poor glycogen formers, show practically the same change in venous as finger blood. Starch produces a much greater reaction in the finger than in the venous blood.

From the above it is apparent that sugar (glucose) curves made on finger blood must always be distinguished from those made on venous blood. Some clinicians have held the view that micro blood sugar estimations made on bloods taken at any time of day were entirely adequate for their purpose. Disregarding the influence of carbohydrate ingestion, such findings should be comparable with those made on venous blood only in severe diabetics who have lost their glycogen forming power.

Estimation of Blood Sugar

A large number of methods have been described for the estimation of blood sugar. Although many of these methods determine the reducing power of the blood (presumably in large part glucose) with a high degree of accuracy, and have given us considerable insight into this phase of carbohydrate metabolism; nevertheless, most of them have been so technical as to require special equipment and training, and are therefore not suited to clinical purposes. S. R. Benedict, who introduced the most satisfactory clinical tests we possess for sugar in urine, has performed, in collaboration with Lewis,[1] a similar service for the sugar of the blood. The general principles and technic of this method are simpler than those of any other method, and for this reason it is particularly well suited to clinical purposes. The method is dependent upon the fact that very small amounts of glucose react with picric acid and sodium carbonate to produce a red color (sodium picramate). In the actual test the picric acid serves the additional purpose of precipitating the proteins of the blood. As has been pointed out by Myers and Bailey,[17] the technic of Lewis and Benedict may be considerably simplified by a lower dilution of the blood, so that the direct picric acid filtrate may at once be used for the colorimetric estimation without evaporation. This method is the one described below. (The Folin-Wu method is given in Chapter IX.)

There has recently been so much discussion of blood sugar methods that some remarks should be made regarding four other methods, which have recently been introduced, and also regarding certain criticisms which have been made of the

picric acid method. In 1920 Höst and Hatlehol[31] presented some very interesting comparative data on the methods of (1) Bang and Hatlehol[32] (Bang's most recent method), of (2) Hagedorn and Jensen,[33] of the (3) Myers-Bailey modification[17] of the Lewis-Benedict method and of the method of (4) Folin and Wu.[34] (The other of the four methods referred to above is the new titration method of MacLean.[35] The recent method of Shaffer and Hartmann[36] is somewhat similar in principle. They employ the Folin-Wu tungstic acid blood filtrate, a copper-carbonate reducing solution and estimate the reduced copper iodometrically.) Höst and Hatlehol find that the first two methods which are titrimetric agree quite closely, but give somewhat lower results than the second two which are colorimetric. This is true of the diabetic bloods in particular, the picric acid method giving the highest results. It is worthy of note that the duplicate determinations with this method were particularly satisfactory. While these differences are theoretically very interesting and will probably be suitably explained, they are so small as to possess little clinical significance in themselves.

Höst and Hatlehol have given the technic of the method of Hagedorn and Jensen which is very neat and requires only 0.1 c.c. of blood. The method of Folin and Wu is adapted to their tungstic acid blood filtrate and requires the equivalent of 0.2 c.c. of blood. In his micro adaptation of their method Foster[30] employs 0.3 c.c. of blood and carries out the color reaction on the equivalent of 0.1 c.c. of blood. Although the Folin and Wu method gives results which are slightly lower than those with the Benedict method, it probably measures the true glucose content somewhat more accurately. It nicely fits their scheme of blood analysis but as an individual determination it requires more time and attention than the picric acid method. They have recently introduced certain improvements.[37]

A number of workers have pointed out that creatinine probably exerted some influence upon the figures for blood sugar obtained with the Lewis-Benedict method, while Morgulis and Jahr[25] have very critically attacked the method on this basis. Criticism was made of results obtained with either the original Lewis-Benedict method or our modification of it, although Morgulis and Jahr employed Benedict's new picric acid reagent, this being much more sensitive to creatinine than the picric acid alone, owing to the presence of hydroxide. In their experiments they found that creatinine gave 3 to 5 times as much color as glucose. As we have pointed out on several occasions (added) creatinine yields the same amount of color with the Myers-Bailey technic as glucose.[38] For example, a blood showing 20 mg. creatinine, and this is rarely encountered, would raise a blood sugar from 0.100 to 0.120 per cent. It is perfectly apparent that this would not account for blood sugars of from 0.15 to 0.25 per cent. Furthermore, as may be noted in Table V, giving the blood findings on eight nephritics with high blood creatinines, there is no relation between the figures for blood sugar and creatinine, low blood sugars being observed with very high creatinines and the reverse.

More recently Behre and Benedict[39] have presented evidence to show that the substance present in blood filtrates which reacts with picric acid and sodium hydroxide in the cold is not creatinine. They have shown, moreover,

that bone-black which removes added creatinine very completely from dilute solutions, such as blood, generally has only a small influence on sugar determinations made by the picrate method on bloods high in "creatinine". This being the case the blood "creatinine" cannot be considered an important source of error in the estimation of blood sugar with the picric acid methods.

Although the Folin-Wu and Benedict methods agree closely in most cases, occasionally blood may be encountered where the latter yields slightly higher results. What the nature of this reducing substance is, it is not possible to say at present. As stated above, the differences are not sufficient, however, to change the clinical interpretation.

Fig. 6.—Myers-Bailey blood sugar tube.

Method[17].—To 8 c.c. of distilled water in a 20 c.c. cylindrical centrifuge tube are added 2 c.c. of the well-mixed oxalated blood (or oxalated plasma). This is then stirred with a glass rod until the blood is thoroughly hemolyzed, after which about 0.5 grams of dry picric acid (sufficient to completely precipitate the proteins and render the solution saturated) is added. The mixture is thoroughly stirred at intervals of several minutes until it is uniformly yellow, after which it is centrifuged and the supernatant liquid filtered into a dry test tube through a small 4 cm. filter paper. (As indicated in the preceding chapter on creatinine, the sugar estimation may be combined with the creatinine, both estimations being carried out on portions of the same picric acid filtrate).

Three c.c. of filtrate are pipetted into a tall, narrow test tube 12 x 200 mm., (sugar tube) graduated to 3, 4, 10, 15 and 20 c.c., 1 c.c. of saturated (22 per cent) sodium carbonate is added, and the tube heated in a beaker of boiling water for 15 to 20 minutes. Simultaneously 3 c.c. of a 0.02 per cent solution of glucose in saturated picric acid (keeps permanently) is treated with a similar amount of sodium carbonate in a sugar tube and heated at the same time as the unknown. This serves as the standard. Under the action of the heat and alkali the yellow sodium picrate is converted to reddish-brown sodium picramate in proportion to the amount of sugar present. The solutions are now cooled to room temperature either by allowing the tubes to stand or by placing them in a beaker of water. The solution in the standard tube is made up to exactly 10 c.c. with water, employing a pipette, preferably with a rubber bulb, so as not to overrun the mark. The unknown tube is now diluted to 10, 15 or 20 c.c., (or some other definite volume in a graduated cylinder) that approximates the color of the standard. It is best to allow a little time to elapse before color comparisons are made.

The standard may conveniently be set at the 15 mm. mark on the colorimeter (Bock-Benedict, Kober or Duboseq). Since the 3 c.c. of blood filtrate employed are the equivalent of 0.6 c.c. of blood and the 3 c.c. of standard solution contain 0.6 mg. of glucose, the proportions are the same as though 100 c.c. of blood and 0.1 gram of glucose were employed.

Calculation.—For the calculation the following formula may be used:

$$\frac{S \times D \times 0.1}{R \times 10} = \text{blood sugar in per cent,}$$

in which "S" represents the depth of the standard (15 mm.), "D" the dilution of the unknown, 0.1 the strength of the standard in grams, calculated on the basis of 100 c.c. of blood, "R" the reading of the unknown and 10 the dilution of the standard.

Estimation of Blood Sugar with the Test Tube Colorimeter

The test tube colorimeter is particularly well adapted to the blood sugar determinations.

Method.—For the blood sugar estimation one may use 3 c.c. of the same filtrate as employed for the creatinine estimation (see page 75). If only the sugar is desired the following technic may be used: 2 c.c. of the well-mixed oxalated blood are treated with 8 c.c. of water (4 volumes) in a 20 c.c. cylindrical centrifuge tube. After the corpuscles have been laked, about 0.5 gm. of dry picric acid is added and the mixture stirred at intervals with a glass rod until it is a light yellow. When the protein precipitation is complete the tube is centrifuged and the supernatant fluid filtered through a small filter paper.

Three c.c. of the filtrate are now pipetted into a test tube (or the right-hand tube of the colorimeter) and 1 c.c. of saturated sodium carbonate (22 per cent) added. To another test tube (or the left hand tube of the colorimeter) add 3 c.c. of 0.02 per cent glucose solution in saturated picric acid (standard) and 1 c.c. of the saturated sodium carbonate. The tubes are now placed in a beaker of boiling water for 15 to 20 minutes, then removed and allowed to cool. The standard is made up to the 10 c.c. mark in the left hand tube with water. The unknown is diluted with water with the aid of the diluting pipette, inverting after each addition, until identical in color with the standard.

Calculation.—Since the equivalent of 0.6 c.c. of blood is employed and 0.6 mg. of glucose is used as a standard, the proportion is the same as though 100 c.c. of blood were used and 0.1 gram of glucose. This being the case, the reading in the unknown tube gives the percentage of blood sugar directly, i.e., a reading of 9 c.c. being equivalent to 0.09 per cent sugar, a reading of 25 c.c. to 0.25 per cent, etc.

Estimation of the Diastatic Activity of the Blood

As indicated above, the diastatic activity of the blood sometimes furnishes information not disclosed by the blood sugar alone. For this reason directions are given for the determination.

Method.[21]—Two 2 c.c. samples of oxalated blood are taken, one being employed as a control. The control tube* is made up to 10 c.c. with distilled water, and the tube to be employed for the test to 9 c.c. Both tubes (20 c.c. cylindrical centrifuge tubes) are now placed in a water bath arranged to maintain a constant temperature of 40° C. As soon as the contents of the tube have been brought to this temperature, 1 c.c. of 1 per cent soluble starch† (or glycogen) is added to the second tube, the contents are mixed, and the incubation carried out for exactly 15 minutes at 40° C. After the incubation has been completed, about 0.5 gram of dry picric acid is at once added to each tube and the mixtures are stirred. When the proteins are precipitated, the tubes are centrifuged and the yellow supernatant fluid is filtered. The sugar in 3 c.c. portions of the filtrate is now estimated as described above for the blood sugar. Correction is made for the sugar originally present in the blood (with the aid of the control) and for the slight reducing action of the soluble starch, if any exists. It has seemed most convenient to record the results in terms of the percentage of the soluble starch (10 mg.) transformed to reducing sugars (calculated as glucose) by the 2 c.c. of blood employed.

Calculation.—The following formula may be used for the calculation of the reducing sugar content of the control (C) and test (A) specimen $\frac{S \times D \times 2.0}{R \times 10}$ = mg. of reducing

*In some cases the incubation of the control results in somewhat higher figures for the blood sugar. It is therefore not always legitimate to take the preliminary blood sugar as the control. It might be desirable, however, to take the increase in the blood sugar on incubation into account in the calculation.

†Many preparations of soluble starch on the market contain reducing sugar. Starch containing much in excess of 6 per cent reducing sugar is not suitable for this work. For some time we have employed soluble starch prepared by the method of Small from potato starch (described on p. 223). This is practically free from reducing sugar. Glycogen is free from reducing sugar and theoretically is to be preferred. Practically, good *soluble starch* gives the same results and is much less expensive.

sugar in terms of glucose for 2 c.c. of blood, in which "S" represents the depth of the standard (15 mm.), "D" the dilution of the unknown in c.c., 2.0 the strength of the standard in mg. compared to 2 c.c. of blood, "R" the reading of the unknown in mm. and 10 the dilution of the standard. The difference between the results of the control and test specimens times 10 gives the percentage transformation of the starch to reducing sugar (diastatic activity), provided the soluble starch requires no correction. For example, a diabetic blood having 0.26 per cent blood sugar would give a control of 5.2 mg. Suppose the test specimen gave 9.8 mg. reducing sugar and the soluble starch contained 6 per cent reducing substance, 9.8 − (5.2 + 0.6) times 10 would give a diastatic activity of 40.

We have also employed the above principle in connection with the Folin-Wu method for blood sugar, using 1 c.c. portions of blood, 0.5 c.c. of soluble starch solution and a 1-5 dilution for incubation. Similar results have been obtained.

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CHAPTER VI

ACID-BASE BALANCE OF THE BLOOD

THAT a condition of acidosis may occur as a secondary complication of a number of pathological conditions has been recognized for some time, while quite recently the possibility of a shifting of the acid-base balance of the body definitely to the alkaline side, resulting in an alkalosis, has received considerable discussion. Normally the hydrogen-ion concentration of the blood is maintained at a very constant level, close to pH 7.4, largely through the protective mechanism of the bicarbonates of the blood. A condition with a normal pH, but with a low blood bicarbonate is spoken of as a compensated acidosis, while a condition with both a low pH and a low blood bicarbonate is termed an uncompensated acidosis.

Although the phenomena of acidosis or acid intoxication would now appear to have been satisfactorily explained, it is only within the past ten years that this has been accomplished. Progress in our knowledge of this subject has been due largely to the development of special methods of blood analysis. Of the different methods suggested to serve as an index of the degree of acidosis, the CO_2-combining power (or content) of the blood would appear to be the most useful clinically, although in any final analysis we are likewise interested in the actual reaction of the blood, i. e., the hydrogen-ion concentration. The introduction by Van Slyke[1, 2] of a relatively simple method of estimating the CO_2-combining power or content of blood plasma has given considerable impetus to the use of this determination as a practical clinical method. It is the object of the present chapter to touch upon only the salient features of acidosis and alkalosis with particular emphasis on this method. For a more detailed discussion, reference may be made to the various papers of Van Slyke and his collaborators on "Studies of Acidosis," especially I[3] and XVII[4]. Van Slyke has also given an excellent review[5] of this important subject.

The term acidosis, although originally coined by Naunyn to apply to the ketosis of diabetes, has come to be used in a much broader sense with the development of our knowledge of this subject. Obviously, acidosis may result either from an abnormal formation of acid substances, such as is found in diabetes, or from a decreased elimination of normally formed substances, as in nephritis. Under conditions of health, the blood is uniformly maintained at a constant slightly alkaline reaction through the influence of the bicarbonate, phosphate and proteins of the blood.

The carbonates of the blood have been called by L. J. Henderson the first line of defense. Increased pulmonary ventilation, as occurs with dyspnea or hyperpnea, serves to increase the excretion of carbon dioxide, thus keeping the

reaction of the blood within normal limits. In conditions of acidosis, other acids may combine with the bicarbonate, robbing the body of its alkali reserve. Under ordinary conditions, however, the kidneys are able to secrete an acid urine from a nearly neutral blood through the medium of acid phosphate, constituting a second means of defense. From the investigations of Marriott and Howland[6] it appears that it is just this factor which breaks down in the acidosis of nephritis. They have found the inorganic phosphates of the blood serum increased to many times the normal in nephritic acidosis, although nephritic cases without acidosis did not show this change. Other means of defense against acidosis are the blood and body proteins, which are able to take up considerable amounts of acids without marked change in reaction, and the ability to form alkali, i.e., ammonia. The latter factor is of considerable importance in the acidosis of such conditions as diabetes and pernicious vomiting, but apparently of little significance in nephritis.[7]

Determination of Degree of Acidosis or Alkalosis

A number of different criteria have been suggested as a measure of the degree of acidosis: (1) lowered carbon dioxide combining power or content of the blood; (2) lowered alveolar carbon dioxide tension; (3) decreased affinity of hemoglobin for oxygen; (4) reduced alkalinity of the blood (Sellards' test); (5) increased hydrogen-ion concentration of the blood; (6) increased intensity of urinary acidity (hydrogen-ion concentration), and (7) the retention of alkali by the body in cases in which the kidney is capable of rapidly excreting an excess of alkali. Yandell Henderson[8] has also suggested the very simple test of holding the breath. A normal person can hold the breath 30 to 40 seconds without an especially deep inspiration, but the period diminishes in proportion to the reduction in the blood alkali. Obviously in the condition of alkalosis there should be an increase in the blood bicarbonate with ultimately some decrease in the hydrogen-ion concentration.

All of these methods have furnished valuable information in the development of our knowledge of this subject, but the first, second, fifth and seventh have yielded information of special clinical value. Although the carbon-dioxide tension of the alveolar air may readily be ascertained with the Fridericia[9] or the Marriott apparatus,[10] the information is not as reliable as the carbon dioxide combining power of the blood determined by the Van Slyke method. That the bicarbonate depletion may roughly be determined by administration of sodium bicarbonate has been pointed out by Sellards,[11] and by Palmer and Henderson,[12] and this method has been in practical use for several years. Normally from 5 to 10 gm. of sodium bicarbonate are sufficient to change the reaction of the urine, but in acidosis of advanced nephritis the deficiency may amount in exceptional instances to as much as 100 gm. of bicarbonate or more.

Palmer and Van Slyke[13] have recently studied this question in connection with the carbon dioxide combining power of the blood. They found that in most pathologic cases the urine did not become more alkaline than blood until a higher plasma bicarbonate had been reached than in normal individuals. This would

result in the giving of unnecessary and possibly injurious amounts of bicarbonate, if the administration were continued until the urine turned alkaline, as has been the usual clinical procedure. The bicarbonate retention may therefore indicate a much more severe acidosis than actually exists. Palmer and Van Slyke advise carefully controlling the therapeutic use of sodium bicarbonate. They have calculated that taking 42 pounds as the unit of weight, 0.5 gm. of sodium bicarbonate will raise the plasma carbon dioxide 1 per cent by volume. In view of this, it is possible to calculate the amount of alkali required to restore the plasma bicarbonate to normal.

Carbon Dioxide Content and Capacity of Whole Blood and Plasma

The normal range for the carbon dioxide combining power of the blood in the adult, as shown by Van Slyke, Stillman, and Cullen,[14] is from 53 to 77 c.c. of carbon dioxide per hundred c.c. of plasma. For normal infants the figures are about 10 c.c. lower than in adults. An idea of the findings for the CO_2 in acidosis of varying degrees of severity may be obtained from Table I.

TABLE I

THE CO_2-COMBINING POWER OF BLOOD PLASMA IN NORMAL SUBJECTS AND IN ACIDOSIS*

CONDITION OF SUBJECT	CO_2 CAPACITY OF PLASMA
	c.c. to 100
Normal resting adult,** extreme limits	77-53
Mild acidosis, no visible symptoms	53-40
Moderate acidosis, symptoms may be apparent	40-31
Severe acidosis, symptoms of acid intoxication	Below 31
Lowest CO_2 observed with recovery	16

*Compiled from Stillman, Van Slyke, Cullen and Fitz.[14]
**The figures for normal infants are about 10 volume per cent lower.

According to Peters and Barr[15] in normal men the total CO_2 content of whole blood under 40 mm. CO_2 tension at 38°C falls between 43 and 55 volumes per cent, about 8 volumes lower than the CO_2 content of the plasma and about 15 volumes per cent lower than the CO_2 capacity of the plasma. As pointed out by Van Slyke and Cullen[3] in their original paper the CO_2 bound chemically with normal human plasma saturated at 20°C with air containing 5.5 per cent CO_2 averages 65 volumes per cent, about 15 volumes per cent more than the total CO_2 content of the whole blood at the time it was centrifuged.

Hydrogen-Ion Concentration of the Blood

Van Slyke[4] has pointed out that the maximum normal range of variation of blood reaction in different individuals appears to be indicated by pH* 7.30 to 7.50, and suggests that it is possible that when errors of technic

*A solution is acid, neutral or alkaline, depending upon the relative concentration of hydrogen ions (H^+) and of hydroxyl ions (OH^-). An acid solution therefore contains a greater concentration of (H^+) than (OH^-).

For convenience in recording the hydrogen ion concentration a simplified logarithmic notation is generally employed, following the suggestion of Sörensen. Pure water, our standard of neutrality, contains $\frac{1}{10,000,000}$ of a gram of H^+ to a liter, and is therefore a $\frac{1}{10,000,000}$ N solution of H. For convenience the logarithmic notation is employed, thus: $\frac{1}{10,000,000N} = \frac{1}{(10)^7N} = 10^{-7}$. Since the base is always 10, and the logarithm always negative the expression is further simplified by drop-

are more completely excluded this range will become still narrower. Our own findings[16] have fallen between pH 7.35 and 7.43. Under extreme abnormal conditions the pH may fall slightly below pH 7.0, and by voluntary deep breathing, on the other hand, carbonic acid may be blown off until the blood alkalinity rises to a pH of 7.7 or 7.8. With the former condition *coma* occurs, while with the latter *tetany* is generally present. Van Slyke suggests that the extreme of reaction compatible with life lies approximately between pH 7.0 and 7.8, and that the normal range is within limits no greater than pH 7.3 to 7.5, and possibly somewhat narrower.

Normal and Abnormal Variations in the Acid-Base Balance of the Blood

Van Slyke[4] has given the possible variations in the acid-base balance of the blood as follows: the blood bicarbonate may be high, low, or normal, and in each of these conditions the pH may be high, low, or normal. There are thus classified nine theoretically possible conditions (see Fig. 7). Only one of these is normal, that in which both bicarbonate and pH are within normal limits.

From the rather limited data available it appears that the pH of various body fluids approximate the blood plasma in their reaction. As there is reason to believe that the CO_2 tension in these fluids approximates that of arterial blood, it appears that a bicarbonate concentration normal for blood plasma indicates also a $[BHCO_3] : [H_2CO_3]$ ratio, and therefore a pH in these fluids normal for blood plasma, (B is employed by Van Slyke to indicate any monovalent base, such as Na or K, $[BHCO_3]$ to indicate the bicarbonate concentration, and $[H_2CO_3]$ the concentration of free carbonic acid.)

As pointed out above the normal CO_2 content for whole blood falls between 43 and 55 volumes per cent at 40 mm. CO_2 tension, and of this Van Slyke and Cullen have shown that approximately 19/20 is in the form of bicarbonate. When the CO_2 tension is other than 40 mm., the normal $[BHCO_3]$ figures also change. Above and below 40 mm. CO_2 tension the normal limits of blood bicarbonate and total CO_2 content rise and fall as indicated by the two absorption curves of Fig. 7, plotted to include an area containing all the apparently reliable data of normal human blood.

If, as pointed out by Van Slyke, a curve is drawn, expressing $[BHCO_3]$ values as ordinates, and H_2CO_3 values as abscissae, the curve will be a straight line for all points corresponding to any given $[BHCO_3] : [H_2CO_3]$ ratio, and the slant will be more or less steep according as the $[BHCO_3] : [H_2CO_3]$ ratio is great or small. But a constant $[BHCO_3] : [H_2CO_3]$ ratio indicates a constant pH (Hasselbalch equation). Quite obviously the pH is a function of the ratio between the $[BHCO_3]$ and the $[H_2CO_3]$. Consequently, one is able by a series of straight, slanting lines on a diagram

ping both the figure 10 and the minus sign. The hydrogen ion concentration of pure water, then, is expressed in terms of its exponent, pH = 7.0. Since the sum of logarithmic expressions H and (OH) ion concentrations is always 14, it will be readily seen that the concentration of either ion may be estimated when one is known. In practice the determination of the hydrogen ion has been found simpler.

For fuller discussion of the pH scale, see Clark, W. M.: The Determination of Hydrogen Ions, 2nd Edit., Baltimore, 1922, 34.

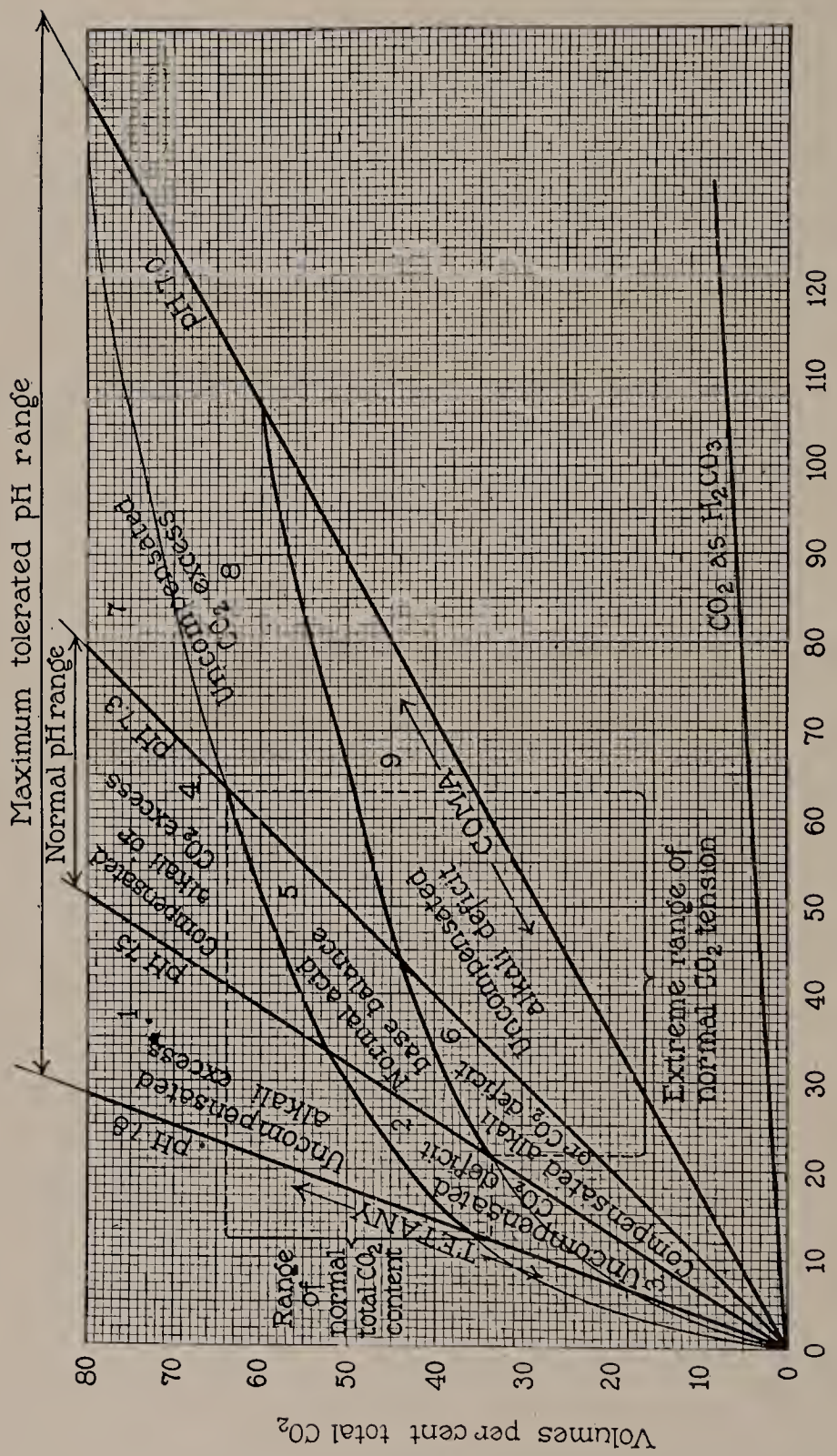

Fig. 7.—Taken from Van Slyke.[4] Normal and abnormal variations of the $[BHCO_3]$, $[H_2CO_3]$, CO_2 tension, and pH in oxygenated human whole blood drawn from resting subjects at sea-level. The bicarbonate CO_2 at any point is obtained by subtracting from the total CO_2 the relatively small amount present as H_2CO_3 indicated by the slanting line near the bottom of the figure.

arranged as given by Van Slyke in Fig. 7 to express all possible $[BHCO_3]$: $[H_2CO_3]$ ratios and pH values.

The diagram given in Fig. 8 is simplified as compared with Fig. 7 by omitting $[H_2CO_3]$ and CO_2 tension values. In this particular diagram the data are for oxalated plasma. No matter whether whole blood or

plasma are employed for the pH determination, the results obtained (electrometrically) appear to be that of the plasma. With the colorimetric method it is the plasma that is actually employed. As pointed out in connection with the methods described below, it is convenient to utilize a portion of the same plasma for its CO_2 content.

In order to ascertain which one of the nine possible variations exists in the blood *in vivo*, it is simply necessary to determine two of the involved variables, such as the pH, $[BHCO_3]$, and $[H_2CO_3]$. With any two of them

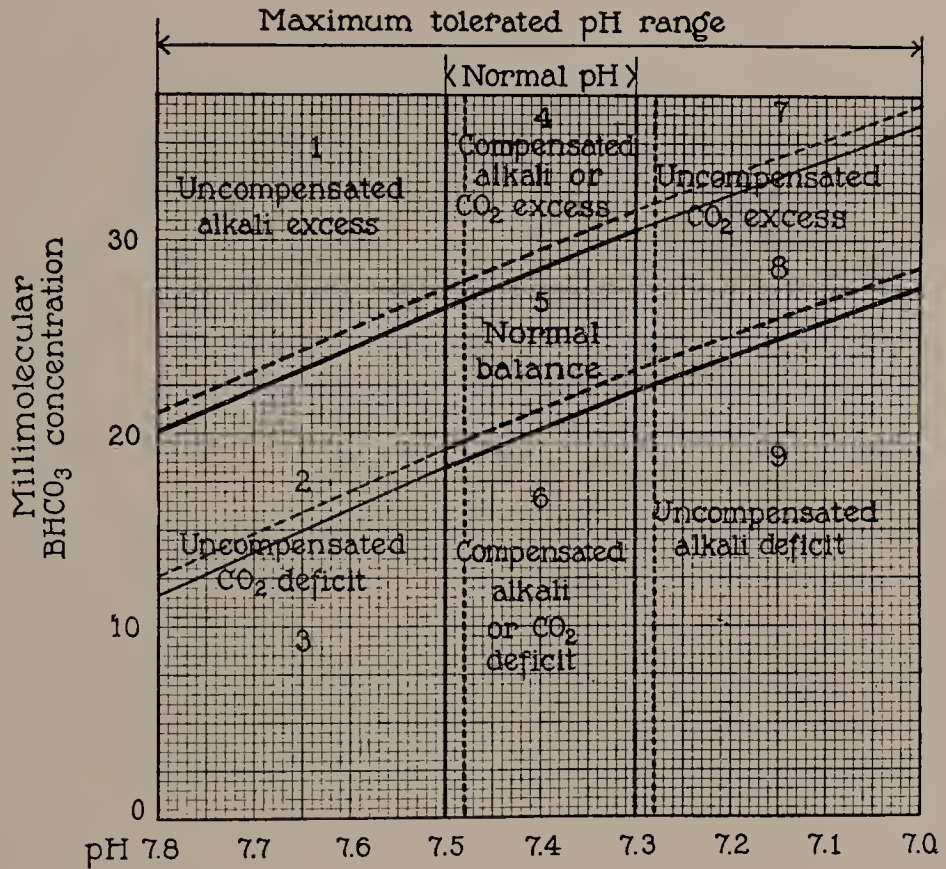

Fig. 8.—Taken from Van Slyke.[4] Normal and abnormal variations of the $[BHCO_3]$ and pH in serum or oxalated plasma. The arterial conditions are indicated by the solid curves, venous by the broken curves. $[BHCO_3]$ and pH values are represented with rectangular coordinates, and the diagram as compared with Fig. 7, is simplified by omitting $[H_2CO_3]$ and CO_2 tension values. $[BHCO_3]$ values are expressed in terms of millimolecular concentration (1 millimolecular $[BHCO_3] = 2.24$ volumes per cent of bicarbonate CO_2). The curves are 4 millimols higher than for whole blood, since $BHCO_3$ concentration in the plasma at any given pH is higher than that of the whole blood by approximately 10 volumes per cent of bicarbonate CO_2, or 4 millimols per liter. The venous bicarbonate curves are only half as far from the arterial in plasma as in the case of whole blood.

a point can be located in its proper area on a diagram such as Fig. 7 or 8. but with any one of them alone it can not be done.

An outline of the various conditions which may fall within the different areas in the diagrams will be given. As pointed out above there are nine possible different conditions of the acid-base balance. Van Slyke[4] has clearly defined the conditions which control these different areas.

Area 1.—Uncompensated Alkali Excess.—In this condition the $[BHCO_3]$, is increased above the normal without a parallel increase in $[H_2CO_3]$. The

result is an increase in the $[BHCO_3] : [H_2CO_3]$ ratio and therefore in pH. This condition may occur after *over dosing with sodium bicarbonate.* It may also occur as a result of loss of gastric HCl due to *pyloric obstruction,* also after *regularly washing out the stomach* for some days. The most marked and characteristic clinical effect of uncompensated alkali excess is the development of symptoms of tetany when the alkalinization proceeds sufficiently far. As Van Slyke has pointed out, one is not at present justified in assuming that all tetany is either caused by, or accompanied by, alkalosis. Greenwald[17] believes that in general the symptoms of tetany observed in so-called alkalosis are due to "sodium poisoning" and that the pH is seldom definitely increased, except in hyperpnea (See Area 2).

Areas 2 and 3.—Uncompensated CO_2 Deficit.—In this condition the $[H_2CO_3]$ is decreased without a parallel fall in $[BHCO_3]$, resulting in an increased pH, due in this case to loss of $[H_2CO_3]$ instead of increase in $[BHCO_3]$. An uncompensated CO_2 deficit has been found to occur in man from *hyperpnea,* either voluntary or where the oxygen content of the air is deficient as at high altitudes. The same condition appears to exist in *fever,* apparently due to the hyperpnea. As a result of various compensatory processes, such retention of acid metabolites and an excretion of bicarbonate, the bicarbonate of the blood may be lowered in some hours from Area 2 to Area 3 (partial compensation), and eventually to Area 6, where the pH is again down to its normal value (complete compensation). The ultimate clinical symptoms at the height of the condition are again those of tetany.

Area 4.—Compensated Alkali Excess or Compensated CO_2 Excess.—Here the pH is normal, as the $[BHCO_3]$ is high but is balanced by a proportionately high $[H_2CO_3]$. With *moderate over doses of sodium bicarbonate* the CO_2 may be retained sufficiently to balance the increased $[BHCO_3]$. Compensated CO_2 excess appears to be the state observed by Scott in *emphysema.* Here the retarded gas exchange presumably leads to a state of chronically increased CO_2 tension in the blood, and the body raises the blood $[BHCO_3]$ high enough to balance the $[H_2CO_3]$ and maintain a normal reaction.

Area 5.—Normal Acid-Base Balance.—The normal area represents the balance that is practically always found in the resting individual at ordinary altitudes.

Area 6.—Compensated Alkali Deficit or Compensated CO_2 Deficit.—Area 6 represents a condition in which the available blood alkali is lowered, but in which a normal pH is maintained (compensated acidosis) because the fall in $[BHCO_3]$ is balanced by a proportional fall in $[H_2CO_3]$. The primary cause of the condition may be a fall in either $[H_2CO_3]$ or $[BHCO_3]$, decrease in the other factor being in each case a secondary balancing or compensatory process, with the apparent physiological object of maintaining a normal pH. Compensated alkali deficit is the condition occurring as the result of accelerated production of non-volatile acids, such as occurs in *diabetes* or their retarded elimination, presumably the case in *nephritis.* The

diarrheal acidoses of infancy also belongs to this group. A fall of blood alkali with maintenance of normal pH may also occur when the primary cause is not acid retention, but excessive respiratory loss of CO_2 (compensated CO_2 deficit). Here the fall in blood bicarbonate is a compensatory process which tends to prevent the blood reaction from becoming abnormally alkaline. Obviously this change may be brought about by the increased breathing necessitated by the *diminished oxygen tension of high altitudes.*

Areas 7 and 8.—Uncompensated CO_2 Excess.—In this condition respiratory excretion of CO_2 is retarded, either by physical hindrance or by deadening of the respiratory center, so that the $[H_2CO_3]$ of the blood is raised. In consequence the $[BHCO_3] : [H_2CO_3]$ ratio and the pH are lowered. This condition has been found to exist after *breathing air rich in CO_2* (3 to 5 per cent) and after the respiratory center has been deadened by *morphine narcosis.* It has also been observed to occur in a *cyanotic pneumonia* patient. Some *cardiac cases* also appear to belong to this group.

Area 9.—Uncompensated Alkali Deficit.—In this condition the $[BHCO_3]$ of the blood is lowered without a proportional fall in $[H_2CO_3]$. In consequence there is a fall in the $[BHCO_3] : [H_2CO_3]$ ratio and the pH, resulting in an "uncompensated acidosis." It has been most frequently observed in cases of *nephritic* and *diabetic acidosis during the premortal period.* In nephritis the uncompensation may occur quite early. In severe diabetic acidosis compensation appears to occur very quickly after the administration of insulin. In *deep ether anesthesia* both alkali deficit and CO_2 retention occur, thus bringing this condition within this area. This combination appears also to occur in *some cardiac cases.*

Data obtained on cases illustrating abnormal variations in the acid-base balance are tabulated in Table II. Cases have been selected which fit into each of the different classified areas in Figs. 7 and 8. It is believed that they excellently substantiate the different conditions laid down by Van Slyke.

Although sodium bicarbonate may apparently be given in fairly large quantity to many individuals without the development of an uncompensated alkalosis, some cases do not well tolerate such administration. This appears to be true in the two cases of cholecystitis with cholecystectomy given in the table, both showing a high pH after alkali administration.

Koehler[18] has recently pointed out that in fever the lung ventilation is apparently sufficiently increased to cause an uncompensated CO_2 deficit and alkalosis. The figures given for the two fever cases (3 and 4) are in harmony with this.

Patients undergoing the Sippy treatment for gastric ulcer appear to be able to take care of the moderate doses of sodium bicarbonate administered. Although the pH in Case 5 is normal, the CO_2 content is definitely high. In Case 6, a child suffering from chorea, the pH is normal, but the CO_2 is high although possibly not abnormal for this age.

The cases showing a normal acid-base balance (normal pH and normal CO_2) require no comment. As indicated by the data on Cases 11 to 14,

TABLE II

CASES ILLUSTRATING VARIATIONS IN THE ACID-BASE BALANCE*

CASE	AGE	SEX	DATE 1923	pH	c.c. to 100 CO_2		DIAGNOSIS, REMARKS
					CONTENT	COMBINING POWER	
AREA 1.—UNCOMPENSATED ALKALI EXCESS							
1. N. M.	52	F	4/9	7.52	83	87	Cholecystectomy, $NaHCO_3$ 40 gm.
			4/10	7.45	79	79	
2. F. H.	41	F	5/3	7.49	76	..	Cholecystectomy, rec'd $NaHCO_3$
AREAS 2 AND 3.—UNCOMPENSATED CO_2 DEFICIT							
3. S. B.	65	F	5/10	7.47	50	..	Fracture, fever (100.8° F.)
4. I. H.	43	M	5/10	7.47	52	..	Syphilis, reaction to arsphenamin, fever (102° F.)
AREA 4.—COMPENSATED ALKALI OR CO_2 EXCESS							
5. G. B.	45	M	7/3	7.40	75	..	Gastric ulcer, rec'd alkali
6. J. S.	9	M	4/13	7.41	63	67	Chorea
AREA 5.—NORMAL BALANCE.							
7. L. C.	26	M	7/4	7.34	69	69	
8. A. S.	60	M	4/13	7.37	..	65	Miscellaneous
9. E. S.	25	M	6/12	7.41	58	59	
10. M. M.	30	M	4/13	7.41	..	67	
AREA 6.—COMPENSATED ALKALI OR CO_2 DEFICIT							
11. E. H.	50	F	7/3	7.36	49	49	Diabetes
12. A. W.	51	M	7/4	7.45	29	34	Diabetes, iletin + $NaHCO_3$
13. M. B.	67	M	6/13	7.36	42	44	Diabetes
14. W. T.	35	M	4/27	7.34	46	..	Chronic nephritis, cardiac, hypertension
AREAS 7 AND 8.—UNCOMPENSATED CO_2 EXCESS							
15. E. L.	47	F	4/20	7.30	63	..	Cardiac decompensation
			4/23	7.40	63	..	Cardiac decompensation; improved
AREA 9.—UNCOMPENSATED ALKALI DEFICIT.							
16. M. I.	37	M	6/4	7.17	13	20	Carcinoma of kidney
			6/8	6.98	4	12	
			6/9	7.01	11	20	Received alkali, died 6/9
17. L. C.	28	M	6/5	7.13	10	13	Diabetes
			6/8	7.31	57	62	Iletin and alkali
18. M. H.	50	F	7/4	7.26	27	29	Nephritis
19. S. B.	7	M	5/8	7.15	..	25	Chronic nephritis
			5/18	7.25	19	..	
			5/25	7.22	20	..	
			5/28	7.16	19	..	
			5/30	7.18	21	..	
			6/1	7.06	17	..	
			6/2	7.05	..	..	Died

*Unpublished observations of Myers, Schmitz and Booher.

cases of diabetes with a moderate lowering of the alkali reserve are still able to maintain a normal pH (compensated acidosis). In nephritis there appears to be considerably greater difficulty in maintaining a compensation and these cases more readily pass from Areas 6 to 9.

As recently pointed out by Peters and Barr[19] in some decompensated cardiac cases the pulmonary and circulatory derangement is sufficient to appreciably influence the CO_2 elimination. This results in a CO_2 excess, and uncompensated acidosis despite the fact that the bicarbonate may be normal. Case 15 belongs to this group. With rest in bed the pH quickly returned to normal.

As pointed out above the premortal cases of diabetes and nephritis with acidosis and the diarrheal acidoses of infancy ultimately pass to Area 9, uncompensated alkali deficit. Our experience would indicate that the larger number of advanced nephritics belong to this group. Contrary to the opinion formerly held, individuals may live for a comparatively long period with low pH values. Case 19 well illustrates this.

Clinical Value of Carbon Dioxide Content or Combining Power

Since pH determinations are not as yet available to the average physician the question will naturally be asked, what are the limitations of the CO_2 content or combining power as an individual determination. As is pointed out below, the chief cause of alkalosis is the administration of over doses of sodium bicarbonate. If the CO_2 of the blood is watched, the dangers of alkalosis can be avoided. In conditions with poor or excessive pulmonary ventilation the bicarbonate of the blood alone may furnish misleading information. All cases with very low CO_2 figures, 20 or under, have an uncompensated acidosis. In cases of diabetic acidosis, showing figures between 25 and 40 the acidosis may be compensated or not. In nephritis, however, the pH seems to fall along with the CO_2. If these facts are properly evaluated, probably the CO_2-combining power will furnish sufficient clinical information in most cases. In the discussion below data on the CO_2-combining power alone are given.

Acidosis in Diabetes

That acetone bodies appear in the urine in large amounts in cases of diabetes with acidosis has long been recognized and their amount taken as an index of the acidosis. Later it was noted that these cases showed a high output of ammonia and this was likewise employed to determine the severity of the acidosis. As indicated above, these findings led to some confusion because they could not be applied to the acidosis of nephritis. The defective oxidation of the fats in diabetes results in the formation of the acetone bodies, acetone, diacetic acid and β-hydroxybutyric acid. The reaction of these organic acids with the sodium bicarbonate of the blood, results in the taking up of sodium by the acids and the setting free of the CO_2, thus robbing the body of its alkaline reserve. What may happen in this respect in diabetes is shown in Table III.

The first two cases died in diabetic coma with CO_2-combining power figures of 12. Case 3 illustrates the findings in a case of severe diabetes, who was still up and about. This patient was referred to the author (as a member of the Medical Advisory Board) as to his availability for the Army, although the fact that he had diabetes was known. Except for shortness of breath and slightly impaired vision, this patient appeared to feel very well. At his own request he was admitted for a short stay in the hospital. The data on Case 4 and 5 illustrate the findings in the average case of diabetes, the latter showing a moderate acidosis. In Case 6 there was a gradual restoration of both the CO_2 and sugar to practically normal.

The body is ordinarily able to handle quite large amounts of acids without

TABLE III

ILLUSTRATIVE FINDINGS FOR THE CO_2-COMBINING POWER OF BLOOD PLASMA IN DIABETES

CASE	AGE	SEX	DATE	CO_2 COMBINING POWER	BLOOD SUGAR	REMARKS
				c.c. to 100	per cent	
1. B. S.	46	♀	8/25/15	12	0.42	Death in coma
2. U. W.	49	♂	2/27/19	25	0.79	Death in coma
			3/ 1/19	12	1.33	
3. A. F.	20	♂	11/12/18	32	0.86	Marked lipemia, Cholesterol 0.64 per cent; left hospital improved
			11/19/18	39	0.41	
4. J. G.	51	♀	6/20/19	59	0.24	Improved
			6/28/19	46	0.24	
			7/ 7/19	55	0.25	
5. R. G.	45	♀	9/11/17	36	0.22	Improved
			9/18/17	52	0.20	
			10/ 9/17	53	0.19	
6. M. B.	27	♀	2/ 9/17	33	0.22	Marked improvement
			2/16/17	45	0.23	
			2/20/17	55	0.19	
			2/27/17	54	0.16	
			3/ 9/17	50	0.18	
			3/27/17	60	0.14	

a marked drop in the alkaline reserve. The tests for acetone and diacetic acid in the urine in diabetes and other conditions are valuable diagnostically, but they tell little concerning the severity of the acidosis. To secure this information it is necessary to resort to such tests as the carbon dioxide combining power of the blood.

Both Allen and Joslin have been opposed to the extensive use of sodium bicarbonate in the treatment of diabetic acidosis, believing that when not severe the condition may be more satisfactorily treated in other ways. Allen states[20] that fasting checks the acetone body formation and that accordingly the great majority of cases of acidosis can be treated by this means alone, and alkali holds no more than a minor adjuvant position. He has shown, however, that occasionally its use is of definite value either in combating a long and stubborn acidosis, or in combating coma in certain severe cases. Here it may save life.

There would appear to be little need for the use of sodium bicarbonate in the treatment of diabetic acidosis at the present time, since as pointed out in the preceding chapter insulin is almost a specific for the acidosis.

Acidosis in Nephritis

Many cases of renal disease show a more or less pronounced acidosis. In a recent report Chace and Myers[21] have concluded that all fatal cases of chronic nephritis with marked nitrogen retention show a severe acidosis, sufficient in many instances to be the actual cause of death. Their experience would lead them to believe that not even moderately severe acidosis was encountered in cases of nephritis without considerable nitrogen retention. Cases of acute nephritis may occasionally show a severe acidosis. To illustrate the importance of acidosis in nephritis, three groups of two cases each are tabulated in Table IV. The first two patients suffered from severe chronic nephritis, showing marked nitrogen

retention and acidosis. It is worthy of note that Case 1 was up and about at the time of the first analyses, while Case 2 after a short stay in the hospital, was at home for a period of seven weeks feeling improved. In both of these cases there was a very severe acidosis, and at the end the CO_2 dropped to such a low level as to be incompatible with life. The observations of Whitney[22] make it evident that such low figures may be the direct cause of death. As these patients did not receive alkali (until after the last recorded test), the CO_2 obviously fell to a lower level than would otherwise have been the case. The findings in these two cases are the lowest we have encountered, the figures more often falling between 20 and 30 during the last days of life. As pointed out above such figures for the CO_2-combining power in chronic nephritis almost always indicate an uncompensated acidosis.

TABLE IV

ACIDOSIS IN CHRONIC AND ACUTE NEPHRITIS*

CASE	AGE	SEX	DATE	CO_2 COMBINING POWER C.C. PER 100	CREATININE, MG. PER 100 C.C. BLOOD	UREA NITROGEN, MG. PER 100 C.C. BLOOD	REMARKS
1. E. M.	39	♂	11/30/15	24	17.5	97	
			12/ 4/15	21	21.5	129	
			12/10/15	26	22.3	132	
			12/17/15	15	24.2	150	
			12/24/15	12	26.7	200	Death in chronic nephritis apparently due to acidosis; no alkali given
2. A. N.	27	♀	3/12/18	31	8.6	97	
			3/26/18	28	12.1	110	
			4/16/18	23	11.2	78	
			5/ 7/18	25	15.2	76	
			5/24/18	12	17.0	148	
3. W. W.	30	♂	2/11/16	23	8.5	55	
			2/23/16	21	12.5	76	
			3/ 7/16	52	10.4	60	Acute exacerbation of chronic nephritis; clinical improvement coincident with rise in alkali reserve; alkali given for a time
			4/ 7/16	54	9.5	39	
			5/10/16	22	9.6	56	
4. J. McC.	56	♂	12/28/15	20	5.1	64	
			1/ 8/16	56	4.5	39	
			1/11/16	58	5.4	39	
			2/ 4/16	40	4.8	36	
5. M. McA.	44	♂	1/25/16	22	4.6	71	
			4/11/16	45	2.7	17	
			5/ 8/17	..	2.4	17	
6. W. C.	49	♂	1/15/16	22	3.5	44	Severe acidosis in acute nephritis with complete recovery
			1/17/16	58	4.1	62	
			1/19/16	56	3.2	53	
			1/28/16	..	1.9	19	
			6/11/18	..	1.8	16	

*Taken from Chace and Myers.[21]

Cases 3 and 4 on admission to the hospital showed pronounced symptoms of acidosis. Although both patients died about one month after leaving the hospital, they showed considerable clinical improvement, this being coincident with the rise in the CO_2-combining power of the blood plasma.

The last two patients were cases of acute nephritis, showing severe acidosis

(very marked dyspnea), but ending in complete recovery. Although this type of case is apparently not frequently encountered, it furnishes an interesting contrast to the preceding two groups. Case 6 was admitted supposedly in "uremic" coma. On estimating the blood creatinine and urea, however, we were surprised at the comparatively slight nitrogen retention, but an estimation of the CO_2 of the blood plasma disclosed the apparent difficulty. Two infusions of sodium bicarbonate, 12 grams each, on the fifteenth and sixteenth produced quite remarkable clinical results, and in less than two weeks the blood findings were normal.

That cases of Asiatic cholera develop a severe acute nephritis and a fairly large percentage, uremia is well known. Sellards[23] has obtained most favorable results by the administration of alkali to these cases, markedly lowering the mortality rate.

It may also be noted here that cases of severe pneumonia frequently show considerable nitrogen retention, and often severe acidosis, as shown by the CO_2-combining power of the blood. We have many observations in substantiation of this.

Acidosis Findings in Children

The subject of acidosis occurring with infantile diarrhea is one that has recently received considerable attention, especially at the hands of Howland and Marriott,[24] Chapin and Pease,[25] and Schloss and Stetson.[26] Howland and Marriott have shown that the acidosis found in many cases of severe diarrhea not of the ileocolitis type is not due to the presence of acetone bodies, but apparently is due to deficient excretion of acid phosphate by the kidneys, as is the case in nephritis. The administration of sodium bicarbonate will often bring about a cessation of the almost characteristic hyperpnea and cause the laboratory tests to give results that are found with normal infants. Nevertheless, the child may die. Schloss and Stetson have applied the Van Slyke method to a quite extensive series of cases. In 27 normal cases the figures ranged from 46

Table V

Cases Illustrating Acidosis in Children*

Case	Age	Sex	Date	CO_2 combining power	Blood sugar per cent	Diagnosis, remarks
	mo.			c.c. to 100		
1. A. N.	2	♀	9/26/15	22	0.09	Gastroenteritis; acidosis; death
2. W. R.	3 1/2	♂	1/20/15	24	0.10	Gastroenteritis; acidosis; death
3. Y. W.	1 1/2	♀	11/ 9/17	24	....	Gastroenteritis; acidosis; death
4. M. K.	10	♂	10/19/17	25	....	Gastroenteritis; acidosis; death
5. J. T.	4	♂	10/13/15	26	0.10	Malnutrition; recovery
6. H. C.	8 1/2	♂	11/15/15	28	0.09	Malnutrition; acidosis; recovery
7. J. M.	12	♀	11/ 3/17	28	....	Gastroenteritis and rickets; vomiting; improvement
8. P. K.	10	♀	11/24/15	33	0.11	Lobar pneumonia; acidosis; death

*Taken from Chapin and Myers.[27]

to 63 c.c. CO_2 per 100 c.c. of plasma, while in 17 out of 19 cases of diarrhea with toxic symptoms his figures ranged from 13 to 38. The acetone bodies of the blood have been studied by Moore.[28] In cases of diarrhea without ileocolitis there is only a moderate increase in acetone bodies, while with ileocolitis the amount of acetone bodies is very large.

Table V presents observations on a few of our cases. It will be noted that the plasma CO_2 ranged from 22 to 25 in the first four cases and that these cases all proved fatal.

Acidosis as the Result of Anesthesia

It is a matter of common observation that urine voided following ether anesthesia gives positive tests for acetone bodies in the urine. More recently it has been shown by Morriss,[29] and others that the anesthesia results in a lowering of the CO_2-combining power of the blood. From this coincidence it was natural to attribute the decreased alkaline reserve to the acetone body formation. Experiments carried out by Short[30] in the author's laboratory indicate, however, that acetone bodies are not formed promptly enough during the anesthesia to account for the decreased plasma bicarbonate. Data are given in Table V on illustrative cases showing the CO_2-combining power of blood plasma before and after ether anesthesia. As will be noted the drop in CO_2 ranged from 4 to 17 volume per cent. From these obser-

TABLE VI

INFLUENCE OF ETHER ANESTHESIA ON THE CO_2-COMBINING POWER OF BLOOD PLASMA*

CASE	AGE	SEX	CO_2 COMBINING POWER c.c. to 100 c.c.		TIME OF ANESTHETIC	OPERATION
			PREOPERATIVE	POSTOPERATIVE		
					min.	
1. E. C.	36	♂	53	49	26	Hemorrhoidectomy
2. W. D.	37	♂	69	60	40	Inoperable carcinoma of stomach
3. E. C.	51	♀	51	45	45	Femoral hernia
4. M. B.	36	♀	54	40	70	Carcinoma of the breast
5. W. C.	50	♂	62	52	43	Inoperable carcinoma of bladder
6. S. A.	40	♀	58	41	43	Right nephrectomy

*Taken largely from Short.[30]

vations it is apparent what would happen if cases of severe diabetes or nephritis with low figures for the CO_2 were operated on under general anesthesia. According to Morriss the drop in the CO_2 is more pronounced after chloroform than ether. A few unpublished observations which have been collected by Killian in this laboratory indicate that the drop in CO_2 is much less after spinal or gas-oxygen anesthesia. Morriss has pointed out that the preliminary administration of sodium bicarbonate increases the alkali reserve, although the most noteworthy effect of this treatment is to lead to higher values for this factor of safety at the conclusion of the anesthetic. Cullen, Austin, Kornblum and Robinson[31] have recently shown that the initial acidosis of anesthesia is an uncompensated one, since here there is an immediate fall in the pH, which precedes the fall in total CO_2.

Alkalosis

Various conditions in which low figures for the blood bicarbonate may be observed have already been discussed. That high figures may be encountered is evident from the data presented in Table VII. Sodium

TABLE VII

CASES WITH HIGH FIGURES FOR THE CO_2 COMBINING POWER*

CASE	AGE	SEX	DATE	CO_2 COMBINING POWER	UREA N	SUGAR	DIAGNOSIS, REMARKS
				c.c. to 100	mg. to 100	per cent	
1. T. A.	4	♂	7/16/21	33	100		Bronchopneumonia, anuria, 30 gm. $NaHCO_3$ intravenously, died.
			7/17/21	125			
2. D. C.	43	♂	11/ 2/22	52		0.374	Diabetes, $NaHCO_3$ given t. i. d. 11/3—11/8, developed bronchopneumonia, anuria, died.
			11/ 5/22	72		0.316	
			11/ 7/22	83		0.284	
			11/ 8/22	100			
			11/10/22	100		0.394	
			11/14/22	81		0.120	
3. N. M.	52	♀	4/ 9/23	98	22	0.227	40 gm. $NaHCO_3$ by rectum following cholecystectomy, improved.
			4/10/23	81	19	0.093	
			4/18/23	49			
4. N. H.	59	♀	11/24/22	98			$NaHCO_3$ by rectum following hysterectomy, coma, died.
5. J. A.	53	♂	8/19/20	64		0.360	Amputation of gangrenous leg, $NaHCO_3$ to 8/21, died 8/23.
			8/21/20	97		0.480	
			8/22/20	80		0.440	
6. T. G.	54	♀	11/17/22	95	29	0.262	$NaHCO_3$ given by rectum for 24 hrs. after cholecystectomy, spasms of facial muscles, coma, pulmonary edema, died.
			11/18/22	75			
7. C. S.	69	♀	7/ 9/18	91	122		$NaHCO_3$ given before admission for gastric ulcer, died, at autopsy carcinoma of pylorus, gall bladder and pancreas.
8. L. G.	70	♂	7/30/18	86	22		Diabetes, amputation of gangrenous leg, $NaHCO_3$ discontinued for 4 days, pulmonary edema, died.
9. R. C.	38	♂	9/29/20	84	70		After 2 days of $NaHCO_3$ by rectum following operation for rt. ureteral and lf. renal calculus.

*Unpublished Observations of Myers and Killian.

bicarbonate had been administered to these cases, some of them, but not all, receiving it in fairly large doses. It might be noted that our high blood bicarbonate figures have been obtained on patients receiving sodium bicarbonate. From the data already presented in Table II we are inclined to the view that probably all of the cases presented in Table VII had a true alkalosis, i. e., high pH, in addition to the high bicarbonate. An attempt was being made to watch closely the blood CO_2 in most of these cases. The data indicate that large doses of sodium bicarbonate should not be administered without a knowledge of the blood CO_2. In fact, the blood CO_2 should serve as the guide to the administration of even moderate

doses of sodium bicarbonate, especially where such therapy is to extend for some time. Judging from the observations of several workers and our own data this is particularly important, when, for any reason, renal function (and consequently the ability to excrete excess alkali) is impaired.

Estimation of the CO_2-Combining Power of the Blood

As already pointed out, the estimation of the carbon dioxide combining power of the blood is probably the most reliable single method of ascertaining the severity of an acidosis or alkalosis. We have long had methods of estimating the CO_2 of the blood, although the subject did not receive much attention previous to the work of Barcroft and Haldane.[32] Many important contributions have emanated from Haldane's laboratory, but it remained for Van Slyke[2] to develop a method so simple that it could readily be employed for clinical purposes. For the extraction of the gas, Van Slyke has made use of a Torricellian vacuum, with which the gas is easily and completely extracted in a closed chamber without any loss. The objection has been raised to the Van Slyke method that determinations made on plasma are not as reliable an index of acidosis as those obtained with whole blood. (The Van Slyke apparatus may be employed for whole blood, but in this case tartaric acid should be substituted for the sulfuric acid). That the CO_2 tension of the alveolar air yields results that are evidently less reliable than the CO_2-combining power of blood plasma is brought out in data presented by Peters.[33]

In the Van Slyke method the plasma from oxalated blood is shaken in a separatory funnel filled with an air mixture, the CO_2 tension of which approximates that of normal arterial blood. In this way it is combined with as much CO_2 as it is able to hold under normal tension. A known quantity of the saturated plasma is then acidified within the gas pipette, and its CO_2 liberated by the production of a partial vacuum. The liberated CO_2 is then placed under atmospheric pressure, its volume carefully measured and the volume corresponding to 100 c.c. of plasma calculated.

As pointed out in connection with the oxygen capacity determination (see page 170) the original apparatus has been improved by Van Slyke and Stadie, while very recently Van Slyke has developed a much more refined ("constant volume") apparatus, whereby the gas is ascertained indirectly by the mm. Hg pressure required to bring it to a volume of 2 c.c. The general principles involved, however, are the same. The water jacketed apparatus, shown an page 169, is quite well suited to clinical use, although the original apparatus may be used.

Method (for CO_2 Content).— For the CO_2 content the blood may be drawn into a glass syringe the air spaces of which are filled with mineral oil (see method for hydrogen ion below), and then delivered under 6 drops of oil into a special centrifuge tube with 5 c.c. bulb. The plasma may readily be separated by centrifuging and conveniently withdrawn with the aid of the special Ostwald pipette shown in Fig. 9. Air is driven out of the small rubber bulb, having a capacity slightly greater than 1 c.c., and the stopcock closed. The pipette is then inserted in the plasma underneath the oil and the stopcock

gently opened. With this manipulation the plasma is drawn into the bulb while the tip of the pipette is in full view and there is no chance of admixture with the cells. The pipette has a double graduation, so that delivery of plasma may be made either between two marks or to the tip of the pipette. The determination of the gas is made in identically the same manner as for the CO_2-combining power, after the equilibration of the plasma with alveolar air.

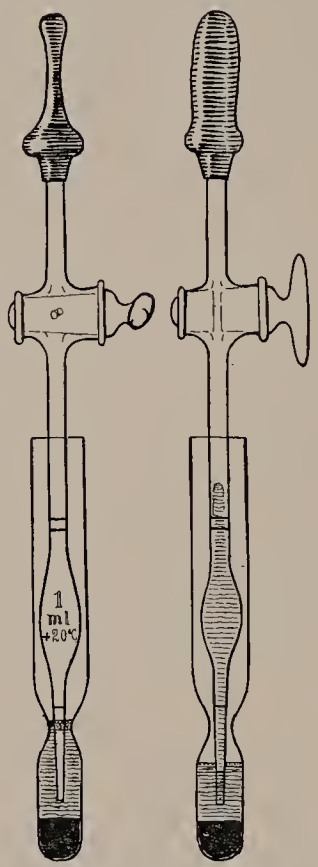

Fig. 9.—Centrifuge tubes and pipette used for the estimation of the CO_2 content of the plasma (author's outfit).

Method[2, 3] *(for CO_2-combining power).*—The blood plasma may be obtained from the blood specimen collected for routine blood analysis as already described (see p. 29) provided a portion of this specimen is centrifuged as soon as secured*. (It is more reliable to collect the blood under oil.) The plasma is then pipetted off. In case the CO_2 capacity cannot be determined at once, the plasma is transferred to a paraffin lined tube, covered with oil, stoppered and placed on ice where it will keep unchanged for a week.

To determine the CO_2-combining power, the plasma is saturated with carbon dioxide at alveolar tension. This can conveniently be done by placing about 3 c.c. of plasma (at room temperature) in a separatory funnel of about 300 c.c. capacity, and the funnel filled with alveolar air from the lungs of the operator. In order to bring the moisture content down to saturation at room temperature, the air is passed through a bottle full of glass beads before it enters the funnel (see Fig. 10). The operator, without inspiring more deeply than normal, expires as quickly and as completely as possible through the glass beads and separatory funnel. The funnel is closed just before the expiration is finished, and is shaken for one minute in such a way that the plasma is evenly distributed about the walls, forming a thin layer which quickly approaches equilibrium with the CO_2 in the air. After the shaking has lasted a minute, a fresh portion of alveolar air is run into the funnel

*Van Slyke and Cullen point out that it is desirable that the subject should avoid vigorous muscular exertion for at least an hour before the blood is drawn, and also best to avoid stasis, or when stasis is necessary to release the ligature as soon as the vein is entered and allow a few seconds for the stagnant blood to pass.

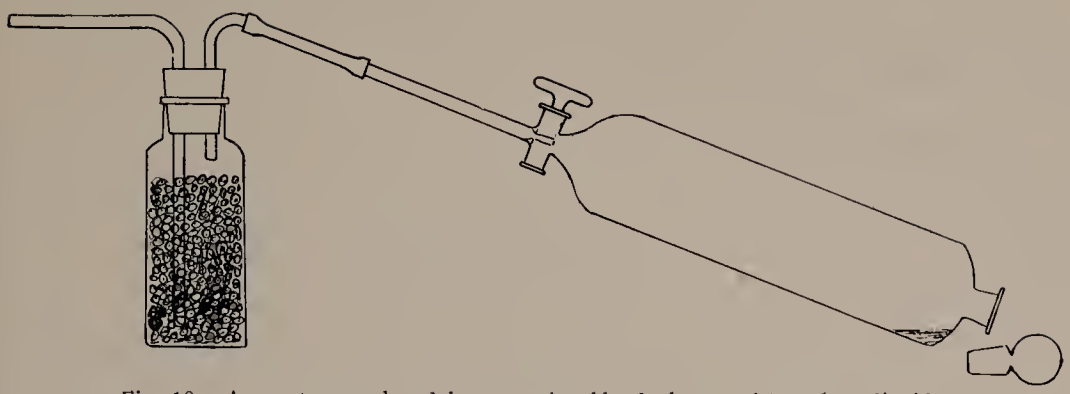

Fig. 10.—Apparatus employed in saturating blood plasma with carbon dioxide.

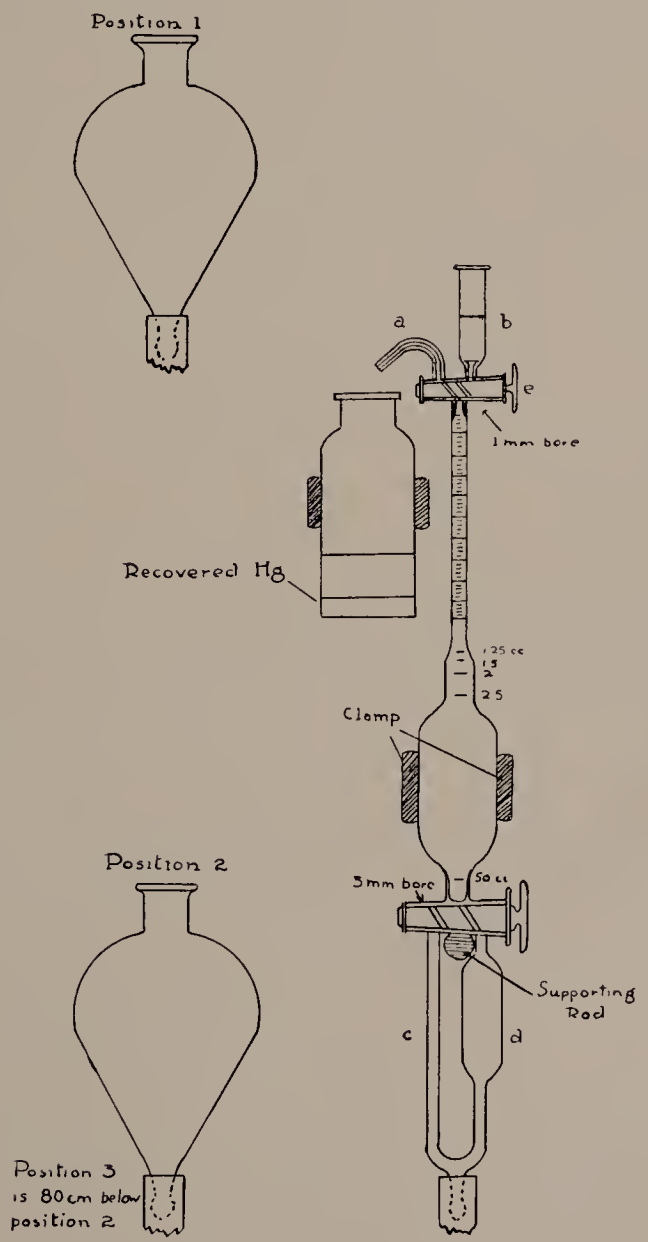

Fig. 11.—Van Slyke gas analysis apparatus.

and the shaking completed. The funnel is now placed in an upright position so that the plasma may drain into the narrow space at the bottom of the funnel and from which it may be readily pipetted.

Technic of Gas Analysis.—The Van Slyke apparatus is illustrated in Fig. 11. It consists essentially of a 50 c.c. pipette with three-way stopcocks at top and bottom, and a 1 c.c. scale on the upper stem divided into 0.02 c.c. divisions. The bottom of the apparatus is connected with a leveling bulb filled with mercury by means of a heavy walled rubber tube. The chamber, *d,* serves to draw off the solutions after the CO_2 has been extracted from them, while *c* serves for the subsequent release of the vacuum by the entrance of mercury. The apparatus is made of strong glass, in order to stand the weight of mercury without danger of breaking, and is held in a strong screw clamp, the jaws of which are lined with thick pads of rubber. To prevent accidental slipping of the apparatus from the clamp, an iron rod covered with a piece of rubber tubing should be so arranged as to project under cock *f* and between *c* and *d*. Capillary *a* is used for convenient removal of solutions from the apparatus. It is of advantage to have a small large-necked bottle clamped to the stand in such a position as to be under the mouth of this tube. Three hooks or rings at the level 1, 2, and 3 serve to hold the leveling bulb at different stages of the analysis. Practically the only source of difficulty with the apparatus is in the entrance of air through the stopcocks. It is essential, therefore that both cocks should be properly greased (a mixture of paraffin and vaseline works very well) and air tight. It is also necessary that the cocks (especially *f*) should be held in place so that they cannot be forced out by pressure of the mercury. For this purpose strong rubber bands work very well.

Before beginning a determination it is necessary to test the apparatus for tightness and freedom from gases. The entire apparatus (Fig. 11), including the capillaries above the upper stopcock, is filled with mercury, and the mercury bulb then lowered to position 3, so that a Torricellian vacuum is obtained, the mercury falling to about the middle of *d*. The leveling bulb is then raised again. If the apparatus is tight and gas-free the mercury will refill it completely and strike the upper stopcock with a sharp click, but in case there is any gas in the apparatus this will serve as a cushion. Should this be the case, the apparatus must be repeatedly evacuated until the gas has all been removed.

For the determination, the apparatus, including both capillaries above the upper stopcock, is entirely filled with mercury by placing the leveling bulb of mercury in position 1, and turning stopcock *e*. The cup at the top is next washed free of acid with carbonate-free ammonia water,* by employing a few drops of the ammonia water, a drop of phenolphthalein and about 1 c.c. of distilled water. This is then all removed but a drop or two, with the aid of a pipette. With an Ostwald-Folin pipette 1 c.c. of the plasma from the separatory funnel is allowed to run into the cup *b* of the apparatus, the tip of the pipette remaining below the surface of the solution in the cup during the transfer, and under the thin film of slightly pink solution. The final drop of plasma is expressed by closing the top of the pipette with the forefinger of one hand, and warming the bulb with the palm of the other.

With the mercury bulb at position 2 and the stopcock *f* in the position shown in the figure, the plasma is admitted from the cup into the 50 c.c. chamber, leaving just enough above the stopcock *e* to fill the capillary so that no air is introduced when the next solution is added. The cup is now washed with two portions of about 0.5 c.c. each of water, care being taken that no air enters the apparatus. A small drop (about 0.02 c.c.) of caprylic alcohol is next added to the cup and permitted to flow entirely into the capillary above *e,* and finally 0.5 c.c. of 5 per cent sulfuric acid is run in. It is not necessary that exactly 1 c.c. of wash water and 0.5 c.c. of acid shall be used, but the total volume of the water solution introduced must extend exactly to the 2.5 c.c. mark on the apparatus if the special formulas of Van Slyke and Cullen in Table VIII are to be used. After the acid has been admitted a drop of mercury is placed in *b* and allowed to run down the capillary

*It is convenient to have a set of five dropping bottles with ground in pipettes and rubber bulbs. The set consists of 1 per cent carbonate-free ammonia water, 1 per cent alcoholic phenolphthalein, distilled water, caprylic alcohol and 5 per cent sulfuric acid.

as far as the stopcock in order to seal the latter. Whatever excess of the sulfuric acid remains in the cup is washed out with a little water.

The mercury bulb is now lowered and hung at position 3, and the mercury in the pipette is allowed to run down to the 50 c.c. mark, producing a Torricellian vacuum in the apparatus. When the mercury (not the water) meniscus has fallen to the 50 c.c. mark, the lower cock is closed, and the pipette is removed from the clamp. Equilibrium of the CO_2 between the 2.5 c.c. of water solution and the 47.5 c.c. of free space in the apparatus is obtained by turning the pipette upside down fifteen or more times, thus thoroughly agitating its contents. The pipette is then replaced in the clamp. By turning the lower stopcock, *f*, the water solution is now allowed to flow from the pipette completely into *d* without, however, allowing any gas to follow it. The leveling bulb is then raised in the left hand, while with the right the stopcock is turned so as to connect the pipette with *c*. The mercury flowing in from *c* fills the body of the pipette, and as much of the calibrated

TABLE VIII

TABLE FOR CALCULATION OF CO_2-COMBINING POWER OF PLASMA*

OBSERVED VOL. GAS $\times \frac{B}{760}$	C.C. OF CO_2, REDUCED TO 0°, 760 mm., BOUND AS BICARBONATE BY 100 C.C. OF PLASMA.				OBSERVED VOL. GAS $\times \frac{B}{760}$	C.C. OF CO_2, REDUCED TO 0°, 760 mm., BOUND AS BICARBONATE BY 100 C.C. OF PLASMA.			
	15°	20°	25°	30°		15°	20°	25°	30°
0.20	9.1	9.9	10.7	11.8	0.60	47.7	48.1	48.5	48.6
1	10.1	10.9	11.7	12.6	1	48.7	49.0	49.4	49.5
2	11.0	11.8	12.6	13.5	2	49.7	50.0	50.4	50.4
3	12.0	12.8	13.6	14.3	3	50.7	51.0	51.3	51.4
4	13.0	13.7	14.5	15.2	4	51.6	51.9	52.2	52.3
5	13.9	14.7	15.5	16.1	5	52.6	52.8	53.2	53.2
6	14.9	15.7	16.4	17.0	6	53.6	53.8	54.1	54.1
7	15.9	16.6	17.4	18.0	7	54.5	54.8	55.1	55.1
8	16.8	17.6	18.3	18.9	8	55.5	55.7	56.0	56.0
9	17.8	18.5	19.2	19.8	9	56.5	56.7	57.0	56.9
0.30	18.8	19.5	20.2	20.8	0.70	57.4	57.6	57.9	57.9
1	19.7	20.4	21.1	21.7	1	58.4	58.6	58.9	58.8
2	20.7	21.4	22.1	22.6	2	59.4	59.5	59.8	59.7
3	21.7	22.3	23.0	23.5	3	60.3	60.5	60.7	60.6
4	22.6	23.3	24.0	24.5	4	61.3	61.4	61.7	61.6
5	23.6	24.2	24.9	25.4	5	62.3	62.4	62.6	62.5
6	24.6	25.2	25.8	26.3	6	63.2	63.3	63.6	63.4
7	25.5	26.2	26.8	27.3	7	64.2	64.3	64.5	64.3
8	26.5	27.1	27.7	28.2	8	65.2	65.3	65.5	65.3
9	27.5	28.1	28.7	29.1	9	66.1	66.2	66.4	66.2
0.40	28.4	29.0	29.6	30.0	0.80	67.1	67.2	67.3	67.1
1	29.4	30.0	30.5	31.0	1	68.1	68.1	68.3	68.0
2	30.3	30.9	31.5	31.9	2	69.0	69.1	69.2	69.0
3	31.3	31.9	32.4	32.8	3	70.0	70.0	70.2	69.9
4	32.3	32.8	33.4	33.8	4	71.0	71.0	71.1	70.8
5	33.2	33.8	34.3	34.7	5	71.9	72.0	72.1	71.8
6	34.2	34.7	35.3	35.6	6	72.9	72.9	73.0	72.7
7	35.2	35.7	36.2	36.5	7	73.9	73.9	74.0	73.6
8	36.1	36.6	37.2	37.4	8	74.8	74.8	74.9	74.5
9	37.1	37.6	38.1	38.4	9	75.8	75.8	75.8	75.4
0.50	38.1	38.5	39.0	39.3	0.90	76.8	76.7	76.8	76.4
1	39.1	39.5	40.0	40.3	1	77.8	77.7	77.7	77.3
2	40.0	40.4	40.9	41.2	2	78.7	78.6	78.7	78.2
3	41.0	41.4	41.9	42.1	3	79.7	79.6	79.6	79.2
4	42.0	42.4	42.8	43.0	4	80.7	80.5	80.6	80.1
5	42.9	43.3	43.8	43.9	5	81.6	81.5	81.5	81.0
6	43.9	44.3	44.7	44.9	6	82.6	82.5	82.4	82.0
7	44.9	45.3	45.7	45.8	7	83.6	83.4	83.4	82.9
8	45.8	46.2	46.6	46.7	8	84.5	84.4	84.3	83.8
9	46.8	47.1	47.5	47.6	9	85.5	85.3	85.2	84.8
0.60	47.7	48.1	48.5	48.6	1.00	86.5	86.2	86.2	85.7

*Taken from Van Slyke and Cullen.[8]

stem at the top as is not occupied by the gas extracted from the solution. A few hundredths of a c.c. of water which could not be completely drained into *d* float on top of the mercury in the pipette, but the error caused by reabsorption of carbon dioxide into the small volume of water is negligible if the reading is made at once. The mercury bulb is placed at such a level that the gas in the pipette is under atmospheric pressure, and the volume of the gas is read off on the scale. In order to have the column in the pipette exactly balanced by that outside, the surface of the mercury in the leveling bulb should be raised until it is level with the mercury meniscus in the pipette, and then, for entire accuracy, raised above the latter meniscus by a distance equal to 1/14 the height of the column of water above the mercury in the pipette.

By means of Table VIII the readings on the apparatus can be directly transposed into c.c. of CO_2 chemically bound by 100 c.c. of blood plasma. This table takes into account the air which enters the apparatus dissolved in the water, etc. The barometer reading and room temperature are taken at the time of the determination. For convenience in the calculation, values are given in Table IX for the ratio $\frac{\text{barometer}}{760}$ over the range usually encountered.

Table IX

BAROMETER	BAROMETER / 760	BAROMETER	BAROMETER / 760
732	0.961	756	0.995
734	0.963	758	0.997
736	0.968	760	1.000
738	0.971	762	1.003
740	0.974	764	1.006
742	0.976	766	1.008
744	0.979	768	1.011
746	0.981	770	1.013
748	0.984	772	1.016
750	0.987	774	1.018
752	0.989	776	1.021
754	0.992	778	1.024

From an inspection of Table VIII it will be observed that the difference between the readings and actual values of CO_2 gas bound is roughly 12. When we first began the use of their apparatus, Drs. Van Slyke and Cullen suggested to us that a correction of 12 would give sufficiently accurate results for clinical purposes, thus eliminating the use of the table or a calculation. We have frequently done this. Somewhat greater accuracy might be obtained by deducting 10 from readings 20-35, 11 from readings 36-40, 12 for readings 51-70 and 13 for readings 71-85.

After the determination has been finished, the leveling bulb is again lowered without opening the upper stopcock, and most of the mercury is withdrawn through *c*. The water solution from *d* is readmitted and, the leveling bulb being raised to position 1, the water solution, with a little mercury, is forced out of the apparatus through *a*. The apparatus is now ready for another determination. It is not necessary to wash it out, since the few drops which remain in it attached to walls hold no measurable amount of carbon dioxide. When not in use the entire apparatus should be filled with water.

Estimation of the Hydrogen-ion Concentration of Blood Plasma

The electrometric method of determining the hydrogen-ion concentration of the blood has been too complicated to permit of clinical use, while until very recently the colorimetric method has not possessed the necessary reliability. Cullen[34] has gone over this question very carefully and suggested a colorimetric method, whereby the hydrogen-ion concentration of blood plasma or serum may be estimated with a high degree of accuracy.

With this method Cullen employs the customary series of tubes with graduated pH values as standards, but Myers, Schmitz and Booher[35] have recently pointed out that the Myers bicolorimeter[36] (See Fig. 33, p. 207) may easily be adapted to this determination. This instrument greatly simplifies the determination and considerably increases its delicacy. By a simple technic contact with air has been entirely excluded. The final determination is carried out on 0.1 c.c. of plasma, and requires less than 10 minutes after the blood has been obtained. The error in color comparison falls within ± pH 0.02.

Method.—Blood is drawn without stasis into a narrow 5 c.c. Luer glass syringe containing sufficient mineral oil to fill any air spaces, and is at once delivered into a centrifuge tube of special design under oil (see Fig. 12). This tube is made of Pyrex glass and has

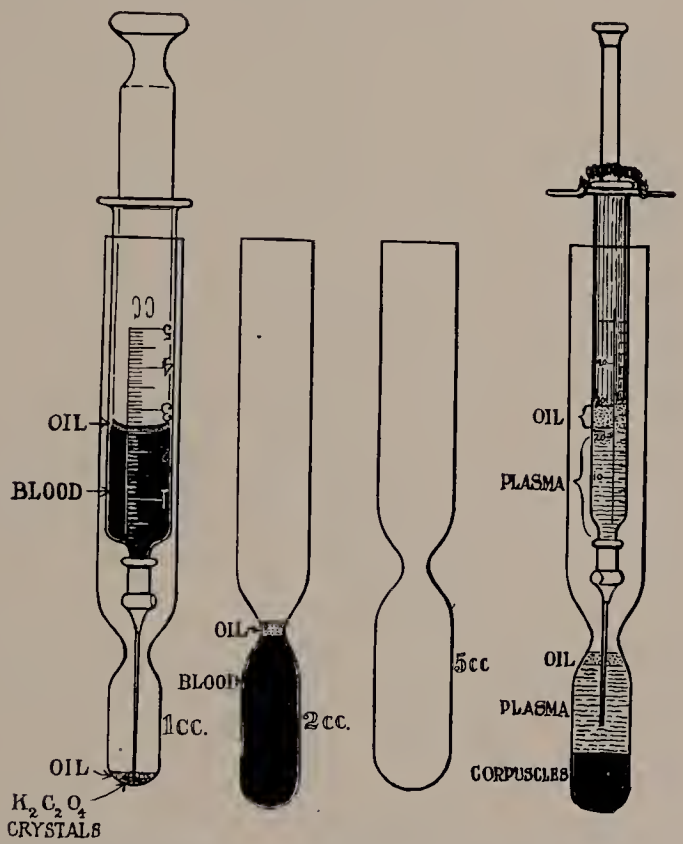

Fig. 12.—Author's apparatus for collection of blood and separation of plasma in the colorimetric pH determination.

at the bottom a bulb of 2 c.c. capacity (30 mm. in length with an internal diameter of 11 mm. and a neck of 4 mm.). Tubes with bulbs of 1 and 5 c.c. capacity may also be used, the latter being employed when a simultaneous estimation of the CO_2 content of the plasma is to be made. One drop of neutral 20 per cent potassium oxalate is dried in the tube, after which three drops of mineral oil are added (six drops in the case of the 5 c.c. tube). In transferring the blood from the syringe to the centrifuge tube, the point of the needle is placed under the oil and sufficient blood delivered to bring the oil into the neck of the bulb. With the slight pressure exerted the blood readily takes up the oxalate and does not clot. The tube is centrifuged at moderate speed for about two minutes to separate the plasma.

A 0.9 per cent solution of sodium chloride in CO_2 free water, to which has been added 10 c.c. of 0.02 per cent phenol red solution for each 100 c.c., is adjusted to a pH of between 7.4 and 7.5 with sodium hydroxide. This can conveniently be done by dip-

ping the tip of a long slender stirring rod in 1 per cent sodium hydroxide, stirring the solution in the flask, and repeating the operation until the solution, when placed in the cup of the colorimeter, gives the correct reading.

There is considerable difficulty in keeping this extremely delicate solution at the correct pH, owing to a tendency for it to become acid. The apparatus, illustrated in Fig. 13, will, in a large measure, overcome this difficulty. The entire apparatus is constructed

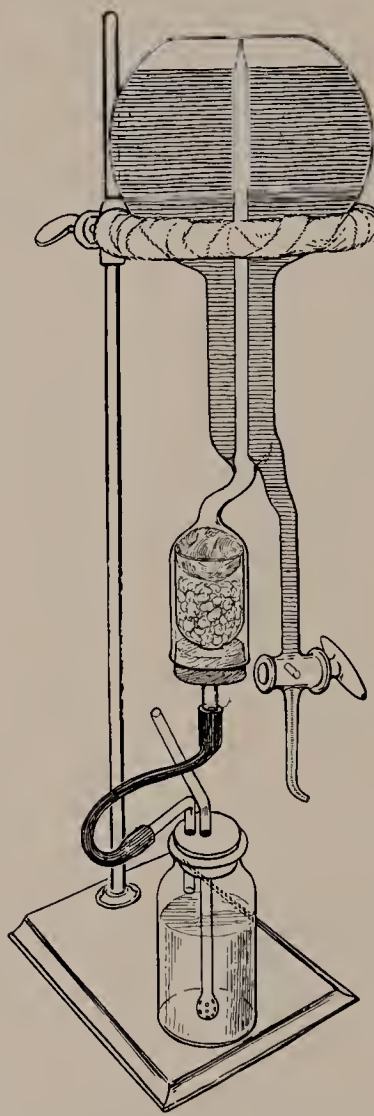

Fig. 13.—Reservoir for saline solution employed in pH determination.

of Pyrex glass, an ordinary liter flask being the starting point. A cylindrical funnel, the stem of which has a small opening (1.5 mm.) passes almost to the bottom of the flask. When the glass stopper is removed, it is possible to quickly fill the flask with the saline. The stopper is inserted, the flask inverted, and any solution in the inverted funnel allowed to drain out. Finally the funnel is wiped out and a tube containing soda lime is tightly inserted. As an added precaution this tube is connected to a small wash bottle containing sodium hydroxide. The flask may also be filled through the stopcock with the aid of suction, after the air in the flask has been freed of CO_2 by passing it through sodium hydroxide. It has been found that when the saline solution is made up at a pH of about 7.55 (to allow for the slight initial drop in the pH, which it seems unable to prevent) the solution may be used for some time without adjustment.

Two c.c. of this saline solution are allowed to flow into the cup of the bicolorimeter under oil. A small portion of the separated plasma is now drawn into the 0.5 c.c. tuber-

culin syringe (see Fig. 12) graduated in 0.01 c.c. (the point of the needle can best be cut off), the air spaces of which are filled with oil; 0.1 c.c. of the plasma is immediately discharged into the saline solution in the cup. This solution is stirred with a small glass rod and is then ready for color comparison.

Calculation.—The factors worked out by Cullen to correct the pH values to body temperature (38° C.) are employed. Cullen recommends that the estimations be made at 20° C., although if this is not feasible, temperatures between 20° and 30° C. may be employed. The following formula, using Cullen's factor of 0.22 as the average correction for colorimetric pH readings in human blood plasma, is used:

$$pH_{38^\circ} = pH_{t^\circ} + 0.01\ (t^\circ - 20^\circ) - 0.22.$$

For example a colorimetric reading of 42 has a pH value of 7.55 on the graph. With a temperature of 24° C. the formula would work out:

$$7.55 + 0.01\ (24 - 20) - 0.22 = 7.55 - 0.18 = \text{pH } 7.37.$$

In instances where the plasma is cloudy the third wedge containing water to which has been added 2 or 3 drops of milk and one drop of formaldehyde may be employed to equalize the field. In this way perfect color matches may always be obtained.

For color comparison the two wedges of the colorimeter are filled with Sörensen's buffer phosphate solutions, containing 2 c.c. of 0.02 per cent phenol red for 20 c.c. of phosphate solution, the front wedge having a

Table X

Phosphate Mixtures (Phenol Red Range)

pH	M/15 Na_2HPO_4	M/15 KH_2PO_4
	c.c.	c.c.
7.0	6.11	3.89
7.1	6.66	3.34
7.2	7.20	2.80
7.3	7.68	2.32
7.4	8.08	1.92
7.5	8.41	1.59
7.6	8.70	1.30
7.7	8.94	1.06
7.8	9.15	0.85

pH value of 8.0 and the second wedge of 6.8. For the alkaline wedge 1.1 c.c. of M/15 primary and 18.9 c.c. of M/15 secondary phosphate are employed, while for the acid wedge 10.2 c.c. of primary and 9.8 c.c. of secondary phosphate are used. For the calibration of the wedges, nine different standards are employed covering the pH range from 7.0 to 7.8, the standards differing by 0.1 pH. The M/15 phosphate solutions should be prepared as described by Cullen from special reagent salts (Merck's are satisfactory) by dissolving 9.47 gm. of the anhydrous Na_2HPO_4 in water and making up to a liter with distilled water. The primary potassium phosphate is similarly prepared from 9.08 gm. KH_2PO_4. Five (or 10 c.c.) portions of the different phosphate mixtures are prepared in Pyrex test tubes according to the data given in Table X and 0.5 (or 1.0) c.c. of the phenol solution added to each tube. The readings made with the wedge containing the dominant color (alkaline, pH 8.0) are employed in plotting the curve from which the calculations are made. Although the solutions in the wedges

apparently keep for a long time, it is best to check them against new standards once a week.

An idea of the type of curve obtained by this method of calibration is given in Fig. 14. The average figures of several repeated standardiza-

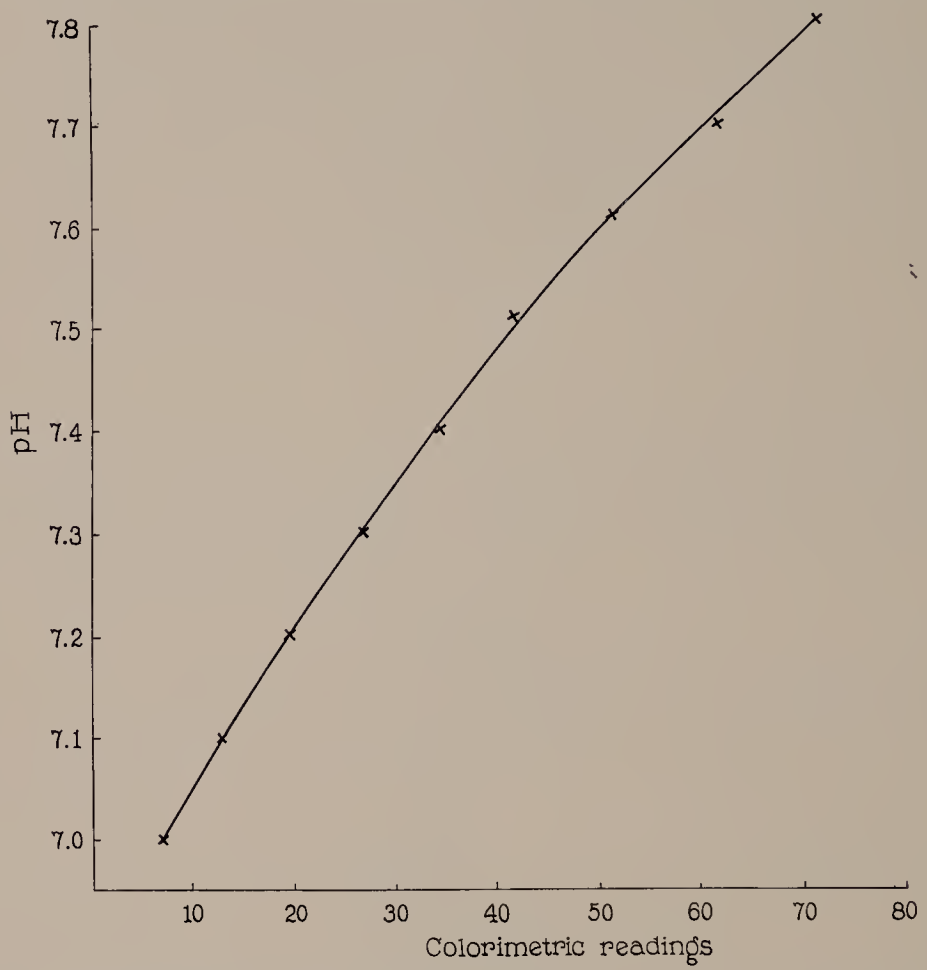

Fig. 14.—Type of curve obtained in the calibration of the alkaline wedge of the bicolorimeter for the pH determination.

tions will give an almost perfect curve, even better than the one in Fig. 14. In making this calibration, one should employ plotting paper having 10 divisions for each 0.1 of pH so that readings in 0.01 pH can easily be made.

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CHAPTER VII

CHOLESTEROL

DURING the past ten years cholesterol has been the subject of many varied and extended investigations, the larger number of which have been carried out on the blood. Although many of these studies have furnished information of great value regarding the physiologic and pathologic rôle of this interesting lipoid, they have disclosed only a few deviations which can be utilized to advantage in diagnosis.

The importance of cholesterol is indicated by its widespread occurrence in the animal body. Cholesterol is obviously, therefore, a constituent of our various animal foods, from which probably much of the cholesterol of the body is derived. According to Gardner,[1] and his coworkers, the phytosterols of the plant foods are transformed to cholesterol in the body, evidently furnishing a portion of our supply of this substance. Whether or not cholesterol is synthesized in the body remains a disputed question. Luden[2] has clearly demonstrated the augmenting influence of animal foods, particularly eggs, butter and meat on the blood cholesterol, while Rothschild and Rosenthal[3] have advocated the use of diets low in cholesterol in the treatment of certain types of cholelithiasis with hypercholesterolemia.

Although cholesterol is a monatomic, simple, unsaturated, secondary alcohol, and further possesses the character of a complicated terpene, its close association with fat and lecithin, and the fact that it may combine with a fatty acid to form a fat makes it of special interest in this connection. Bloor,[4] in particular, has given us much useful information regarding the variations in these three lipoids in the blood. Normally the "total fat" content of the blood plasma amounts to 0.6 to 0.7 per cent, but in the severe lipemia of diabetes figures as high as 26 per cent have been observed. The cholesterol content of the blood runs fairly parallel with the total fatty acids in all cases, including lipemia, and on this account is an excellent index of the degree of lipemia in diabetes. Cholesterol occurs in the blood in both the free and combined state. Free cholesterol is present in the corpuscles and to some extent in the plasma, and the cholesterol esters in the plasma alone. Bloor and Knudson[5] have found that in whole blood the average percentage of cholesterol in combination as esters is about 33.5 per cent, and in the plasma 58 per cent of the total cholesterol. Most of the data recorded in the literature, however, are for the total cholesterol of the blood, some of the results being on the plasma or serum, others on the whole blood. Normally, the concentration of cholesterol is nearly the same in the plasma and the whole blood, although, if anything, the plasma content is slightly higher, and pathologically it seems to be subject to somewhat greater variations. That such is the case is well illustrated in the following Table I abbreviated from Grigaut.[6]

TABLE I

DISTRIBUTION OF CHOLESTEROL IN THE BLOOD*

CONDITION	CHOLESTEROL IN PER CENT			
	SERUM	PLASMA	WHOLE BLOOD	CORPUSCLES
1. Normal man	0.168	0.168	0.159	0.141
2. Normal man	0.170	0.170	0.150	0.130
3. Normal woman	0.174	0.170	0.168	0.171
4. Normal woman	0.175	0.175	0.165	0.140
5. Carcinoma of the pancreas with jaundice	0.071	0.068	0.105	0.110
6. Pneumonia	0.098	0.098	0.110	0.150
7. Carcinoma of the liver with jaundice	0.222	0.228	0.198	0.170
8. Diabetes	0.246	0.246	0.201	0.137
9. Cholelithiasis	0.276	0.270	0.225	0.180
10. Nephritis	0.450	0.450	0.285	0.150
11. Nephritis	0.514	0.514	0.264	0.135
12. Carcinoma of the pancreas with jaundice	0.840	0.840	0.540	0.195

*Taken from Grigaut.[6]

Chauffard, Laroche and Grigaut[6] have given 0.15 to 0.18 per cent as the normal value for the cholesterol content of blood serum, figures which closely agree with the findings of subsequent workers. This would make the figures for whole blood about 0.14 to 0.17 per cent. Pathologically, many conditions have been recorded in which a hypercholesterolemia was found, while in a few conditions hypocholesterolemia has been noted. In general it may be stated that hypercholesterolemia is found in arteriosclerosis, nephritis, nephrosis, diabetes (especially with acidosis), obstructive jaundice, in many cases of cholelithiasis, in certain skin diseases, in the early stages of malignant tumors, and in pregnancy. The chief condition in which low values for cholesterol are found is anemia.

The one disorder in which cholesterol appears to be of definite diagnostic value is that group of chronic parenchymatous nephritis cases to which the name of nephrosis has been given. Although Grigaut early discovered that certain nephritics gave high figures for the blood cholesterol, we owe especially to Epstein[7] recognition of the fact that typical nephrosis is associated with marked hypercholesterolemia.

The increase in the lipoid content of the blood of chronic nephrosis would appear to be of fundamental importance. It is undoubtedly of greater significance than the lipoidemia of diabetes. Although the origin of the lipoidemia in nephrosis is still obscure, it is unquestionably of a different order from that which occurs in diabetes mellitus.

Epstein[7] has well summarized the important diagnostic points in nephrosis (pure form):

> We have then in the case of nephrosis a clinical picture characterized by a gradual onset, a protracted course, edema, anasarca and effusion of the serus cavities; by oliguria, marked albuminuria and occasional cylindruria, a high specific gravity which indicates ability to concentrate the urine. It is also characterized by the absence of any increased blood pressure and any cardiac hypertrophy and by the absence of any marked nitrogen retention in the blood. It is also characterized fundamentally by a marked increase in the cholesterol content of the blood, by a reduction in the total protein of the serum and by the inversion of the normal ratio of albumin to globulin. The whole presents a metabolic disturbance of nutrition. It is more pronounced in younger individuals.

In the treatment of this condition Epstein advocates a diet high in protein, but low in carbohydrate and very low in fat.

Schwartz and Kohn[8] have recently presented data on seventeen cases of nephrosis in children with cholesterol and other blood findings. That the condition is more common in children than in adults is borne out by our own series of cases, observations on some of which are given in Table II. These

TABLE II

CHOLESTEROL AND OTHER BLOOD FINDINGS IN NEPHROSIS*
(All analyses made on whole blood.)

CASE	AGE	SEX	DATE	CHOLESTEROL	CHLORIDES AS NaCl	UREA N	SUGAR	REMARKS, OUTCOME
				per cent	per cent	mg.	per cent	
1. H. P.	3	♀	7/21/21	0.334	0.500	23	0.121	Ascites and edema,
			7/29/21	0.436		23		died after leaving
			10/21/21		0.488	10	0.122	hospital.
2. V. L.	3	♀	8/22/22	0.704		11		Edema, somewhat
			8/24/22	0.600	0.550	13		improved clinically.
			10/10/22	0.440	0.500	22	0.102	
			11/15/22			16		
			1/ 3/23	0.436	0.513	21		
			2/27/23	0.184	0.475	11		
			5/11/23	0.305				
3. E. C.	4½	♂	9/15/22	0.830	0.525	11	0.114	Marked proteinuria,
			10/10/22	0.246	0.500	16	0.096	marked edema, died.
4. S. B.	6	♂	6/21/21	0.499	0.475	11	0.115	Ascites, edema,
			6/23/21	0.507	0.513			twice discharged
			9/16/21	0.540				improved, creatinine on 5/11, 11
			10/21/21	0.227				mg. and CO_2 20,
			5/ 9/23	0.256	0.625	89		died.
			5/11/23	0.241	0.625	98		
5. F. S.	12	♀	3/19/23	0.249	0.538	10		Acute nephritis, discharged improved.
6. B. V.	13	♂	10/31/22	0.332	0.450	12	0.107	Ascites, edema, left
			2/24/23	0.374	0.475	11		somewhat improved.
			3/20/23	0.386	0.488	7		
7. M. T.	27	♂	8/29/21	0.424	0.513	14	0.122	Generalized edema, left unimproved.

*Unpublished observations of Myers and Killian.

cases at some time all disclosed marked proteinuria, marked edema and marked hypercholesterolemia. It will be noted, however, that there is no marked abnormality in the case of the whole blood chlorides, urea nitrogen or sugar, except in case of S. B. This patient, two years after the onset, now appears to be in the terminal stages of chronic nephritis with nitrogen retention, acidosis and salt retention. With this exception the absence of high figures for the blood chlorides, despite the marked edema, is very striking.

Figures for the cholesterol content of whole blood, which are intended to be representative of the findings in the various pathologic conditions mentioned above, excepting nephrosis, are given in Table III. The figures are taken partly from Gorham and Myers[9] and partly from some of our more recent observations. Grigaut[6] has presented a most excellent discussion of the cho-

lesterol content of blood, while the papers of Bloor[4] (include data on other lipoids), Denis,[10] and Gorham and Myers[9] all review this general subject, including the literature. Allen[11] has likewise given a very useful summary of the literature on the blood lipoids.

Rothschild and Wilensky[12] give an excellent outline of the factors influencing the blood cholesterol:

1. The cholesterol content of the blood is lowered:
   (a) By a diet which is poor in lipoids.
   (b) By the occurrence of high temperatures.
2. The cholesterol content of the blood is increased:
   (a) By a diet excessively rich in lipoids.
   (b) By the presence of other diseased conditions, especially diabetes, arteriosclerosis, and nephritis.
   (c) During pregnancy. This lasts for a variable period after evacuation of the uterus.
   (d) By the presence of obstruction in the common bile duct. If the obstruction, however, is not absolute, as indicated by the degree of accompanying jaundice, the cholesterol content of the blood may not be increased.

TABLE III

THE CHOLESTEROL CONTENT OF THE BLOOD IN VARIOUS PATHOLOGICAL CONDITIONS

CASE	AGE	SEX	CHOLESTEROL	CO_2 COMBINING POWER	SUGAR	UREA N	CONDITION
			per cent	c.c. to 100	per cent	mg. to 100 c.c.	
1. H.B.	20	♂	0.82	21	0.39		Diabetes, Case 1 showing a total fat content of 7.1 per cent.
2. A.F.	20	♂	0.63	31	0.86		
			0.57	38	0.41		
3. U.W.	49	♂	0.20		0.79		
			0.25	12	1.16		
4. H.R.		♂	0.34	35	0.30		
5. W.A.	45	♂	0.11	50	0.25		
6. W.F.	25	♂	0.34			100	Bichloride poisoning
7. E.E.	30	♀	0.28			33	Nephritis
8. E.B.	24	♂	0.28				
9. I.D.	17	♀	0.22			170	
10. J.W.	34	♂	0.16			63	
11. M.H.	71	♀	0.23				Arteriosclerosis
12. L.W.	62	♀	0.21				
13. D.G.	23	♀	0.24				Moderately severe pellagra
14. H.C.	44	♀	0.13				Cholelithiasis, confirmed by operation, no jaundice
			0.16				
15. W.L.	15	♀	0.18				
			0.21				
16. F.T.	21	♂	0.21				Catarrhal jaundice
17. R.K.	51	♂	0.29				Obstructive jaundice
18. H.H.	47	♂	0.20				Carcinoma of stomach, early
19. M.L.	45	♂	0.12				Carcinoma of stomach, late
20. L.M.	34	♀	0.23				Pregnancy, 8 mos., eclampsia
21. O.G.	48	♂	0.07				Pernicious anemia, plasma 0.065%, washed cells, 0.12% plasma, 0.065%, washed cells, 0.13%
22. F.S.	50	♂	0.07				

Although the observations recorded in Table III are given only on whole blood, with the exception of the cases of anemia, it is apparent from the data of

Table I that greater variations take place in the cholesterol of the serum or plasma. Nevertheless a significant change in the cholesterol is readily evident from observations made on whole blood.

A more marked hypercholesterolemia may be found in the lipemia of diabetes than in any other condition. The lipemia of the first two cases of Table III, showing cholesterol figures of 0.6 to 0.8 per cent, is very marked. Although the ordinary case of diabetes at the present time does not show lipemia in the sense that the blood is milky, still the lipoids of the blood are increased in all types of the disease. Joslin, Bloor and Gray[13] found that the average quantity of lipoids in the whole blood with the Bloor method amounted to 0.59 per cent in 19 normal individuals, but was increased to 0.83 per cent in 30 mild diabetics, to 0.91 per cent in 37 moderately severe diabetics, and to 1.41 per cent in 55 severe cases of diabetes. This holds for all three groups of lipoids. They state, "The increase in cholesterol is significant and suggestive, and seems indeed pathognomonic of the prolonged diabetic hyperlipemia, since Bloor has found it lacking in the acute lipemia of overfeeding which is characterized by an increase in the total fatty acid alone." On this account the determination of the cholesterol alone should give valuable information regarding the lipoid content of the blood in diabetes.

Although many observers have noted and studied the hypercholesterolemia of nephritis, it is not possible as yet to give a satisfactory interpretation of these findings. In the case of arteriosclerosis, however, it is worthy of note that histologic changes have been observed in the aorta after the experimental administration of cholesterol.

Since gallstones are largely composed of cholesterol, it is reasonable to suppose that their appearance might be associated with an increase in the cholesterol content of the blood. Henes[14] has maintained that this is the fundamental and primary factor in the formation of gallstones. Although it seems quite probable that a hypercholesterolemia is present during the early period of the formation of the calculi, analytical data show wide variations in the blood cholesterol,[3, 9, 12] the findings ranging from low normals to figures that are definitely increased. Rothschild and Rosenthal[3] emphasize the fact that in a certain group of cases the hypercholesterolemia is very persistent and operation affords only temporary relief. They believe that properly selected low cholesterol diets have been very helpful in these cases.

It is logical to expect that in obstructive jaundice the cholesterol content of the blood should be elevated and bear a fairly definite relation to the intensity of the icterus. Rothschild and Felsen[15] have shown, however, that in conditions associated with hepatic disorders the cholesterol of the blood is not increased, but usually reduced, while in so-called hemolytic icterus, there is no increase of blood cholesterol.

It would appear that in the early stages of malignancy, the blood cholesterol was somewhat elevated or normal, while in the late stages the figures are below normal. Luden[2, 16] has called attention to the fact that a diet which increases the blood cholesterol coincidently weakens the lymphoid defense. She suggests that in persons predisposed to carcinoma an increase of the cholesterol and a

weakening of the lymphoid defense, such as may occur with the prolonged use of a high cholesterol diet, may perhaps result in the development of carcinoma.

The hypercholesterolemia of pregnancy is well known. Chauffard, Laroche and Grigaut[17] found that the increase begins about the fourth month of pregnancy and becomes progressively greater as full term is approached. Slemons and Curtis[18] have made the interesting observation that cholesterol esters are frequently absent from fetal blood. In this case the cholesterol is exclusively in the free form, and furthermore, the free cholesterol of both maternal and fetal blood are identical. Apparently the normal placental partition is permeable for free cholesterol but impermeable for cholesterol esters.

That the cholesterol of the blood (plasma) is lowered in anemia has been recognized for some time. When the antihemolytic action of cholesterol is recalled, it will be seen that this observation may possess some practical significance. The therapeutic administration of cholesterol in this condition has received attention from Italian investigators and has apparently been followed by beneficial results. Pacini[19] (in this country) has recently presented some interesting observations on the blood cholesterol in pernicious anemia, giving data on the whole blood, serum and cells. He found the cholesterol markedly decreased in the serum but relatively increased in the cells. He administered cholesterol in the form of lanolin as an inunction, and believed that he obtained definite benefit.

Estimation of Cholesterol

Cholesterol was one of the first constituents of the blood to be determined colorimetrically. Several years before the development of the colorimetric methods of blood analysis described in the previous chapters, Grigaut[6, 20] had already described (1910) a colorimetric procedure of estimating the cholesterol content of blood. In the development of the color Grigaut made use of the Liebermann-Burchard reaction, and the technic of this part of the test is still carried out essentially as he originally described it. Two years after the publication of Grigaut's method, Weston[21] described a procedure in which the Salkowski color reaction was employed. The Liebermann-Burchard reaction is better suited, however, for use in this connection. In 1913 Autenrieth and Funk[22] described a slight modification of the Grigaut technic and adapted it to use with the Autenrieth-Königsberger (Hellige) colorimeter. This modification has been extensively employed and many references may be found to work carried out with the Autenrieth-Funk method. It would seem only fair to Grigaut, however, that this method should bear his name. A number of different workers have described procedures of cholesterol extraction upon which the colorimetric method of Grigaut is applied.

In the case of the excellent but laborious gravimetric digitonin method of Windaus[23] for the estimation of total cholesterol, saponification of the cholesterol esters is necessary, since only the free cholesterol is precipitated by the digitonin. Cholesterol esters give the color reaction as well as does the free cholesterol. This fact does not appear to have been recognized until recently, since the directions for the colorimetric estimation have almost invariably called

for a preliminary saponification. As pointed out by Bloor,[24] this saponification is unnecessary and the colorimetric estimation of the cholesterol thus becomes further simplified.

Bloor has suggested a method of extraction[25] for the cholesterol (and other lipoids) which is very simple and would appear to be complete, but the results obtained with the method as finally carried out (second method) are higher than those by the older methods, and rather irregular, owing apparently, to the presence in the extracts used of substances interfering with the Liebermann-Burchard color reaction for cholesterol. These high results have been criticized by Mueller[26] and Weston,[27] who are of the opinion that they are due to the admixture of brownish tints frequently obtained in the final development of the color. Luden[28] obtained similar high findings with Bloor's second method but believed that these resulted from a combination of bile pigment and bile acids. With Bloor's first method the alcoholic ether extract was saponified with sodium ethylate, this procedure being omitted with the second method. Luden's data bearing on this point are very interesting. Our observations on this subject are in harmony with her conclusions.

Myers and Wardell[29] have described a comparatively simple method of direct cholesterol extraction which appears to yield reliable results. At any rate added cholesterol may be quantitatively recovered, and good checks obtained with the totally different Windaus method. With this method 1 c.c. of blood is mixed with plaster of Paris and dried. In addition to putting the blood into a finely divided and readily extractable condition, this calcium salt apparently holds back substances which add to the color development with the Bloor technic. After reaching its greatest intensity the cholesterol color fades rather rapidly and for this reason Myers and Gorham[30] suggested the use of naphthol green B as a standard. This dye excellently matches the cholesterol color and appears to be permanent.

In carrying out the color reaction it is essential that the reagents should be anhydrous. Poor acetic anhydride will give a weak color development. For this reason it is desirable before developing a series of unknown solutions to first check the quality of reagents by developing a solution of pure cholesterol.

Method.[29]—For the determination, 1 c.c. of blood, plasma or serum is pipetted into a porcelain crucible or small beaker containing 4 to 5 gm. of plaster of Paris, stirred, and dried, preferably in a drying oven for an hour. It is now emptied into a small paper extraction shell (4 cm. long) and then inserted in a short glass tube* (2.5x7 cm.) in the bottom and sides of which are a number of small holes (Fig. 15). This is now attached to a large cork on a small reflux condenser and the tube and cork inserted in the neck of a 150 c.c. extraction flask containing about 20 to 25 c.c. of chloroform. (We have frequently run 3 to 6 extractions simultaneously on the same hot plate.) Extraction is continued for 30 min., on an electric hot plate, the chloroform made up to some suitable volume, such as 20 c.c., filtered if necessary, and colorimetric estimation carried out as follows: 5 c.c. of the chloroform extract are pipetted into a dry test tube, and 2 c.c. of acetic

*Originally we made these tubes from large test tubes by drawing out the tubes, blowing new bottoms (round) and then punching a number of holes with a white hot, malleable iron wire. Later we tried alundum thimbles, in which two small holes had been drilled near the top to easily permit the entrance of the chloroform vapor. Although this obviated the necessity of paper extraction shells, cups could not be found that were sufficiently porous to make a rapid extraction possible. The flat bottom tubes illustrated are an advantage in that they will stand, and the thimble cannot be sucked tight against the bottom of the tube.

anhydride and 0.1 c.c. of concentrated sulfuric acid (best with 0.1 c.c. pipette) are added. After thorough mixing, the solution is placed in the dark for exactly 10 min.* to allow the color to develop, and then compared with a standardized 0.005 per cent aqueous solution of naphthol green B in a Bock-Benedict or Klett colorimeter. If the Duboscq colorimeter is used, it is necessary that the cups should be remounted in plaster of Paris, instead of balsam.

With a good grade of acetic anhydride, it has been found that when an 0.005 per cent solution of naphthol green B is used as a standard and set at 15.5 mm. on the Duboscq

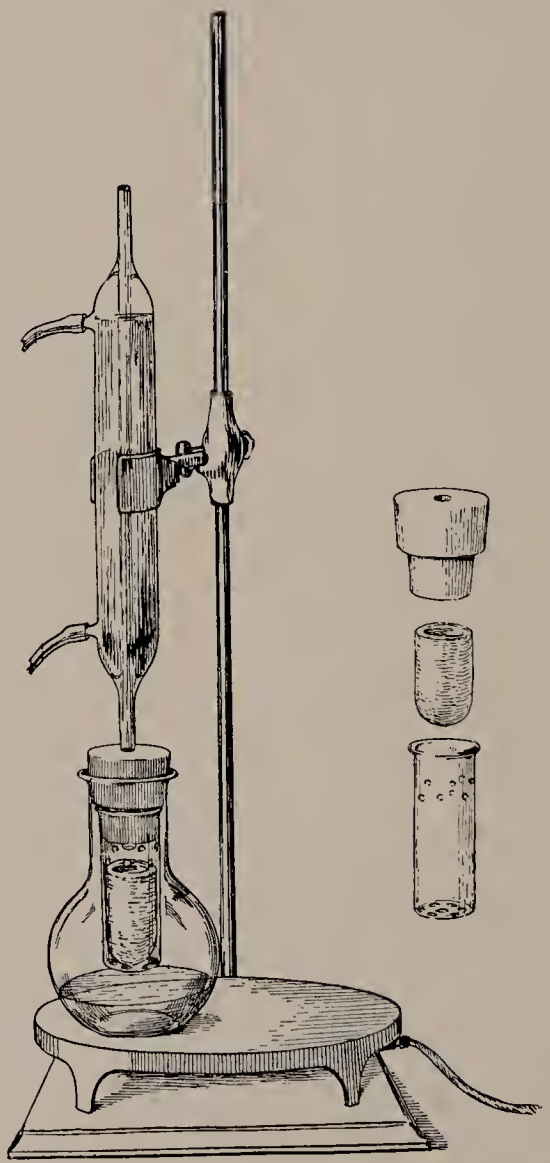

Fig. 15.—Cholesterol extraction apparatus.

or Klett instrument, 0.4 mg. of cholesterol in 5 c.c. of chloroform treated with 2 c.c. of acetic anhydride and 0.1 c.c. of concentrated sulfuric acid will read 15 mm. The color curves for both the cholesterol and naphthol green B appear to fall in a straight line so that readings somewhat above or below the standard are accurate.

Calculation.—If a cholesterol standard containing 0.4 mg. to 5 c.c., or a naphthol green

*In order to get the proper temperature for color development in warm weather it is advisable either to keep the reagents in a cool place or to insert the tubes in water during the development of the color.

B standard of equivalent strength, are employed, the following formula may be used for the calculation: $\frac{S}{R} \times 0.0004 \times \frac{D}{5} \times 100 =$ cholesterol content of blood in per cent, in which S stands for the depth of standard in mm., R for the reading of the unknown, 0.0004 the equivalent amount of cholesterol in 5 c.c. of chloroform, D the dilution of the chloroform extract from the 1 c.c. of blood, 5 the dilution of the standard and 100 the factor for 100 c.c. For example: $\frac{15}{15} \times 0.0004 \times \frac{20}{5} \times 100 = 0.160$ per cent.

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CHAPTER VIII

CHLORIDES

ALTHOUGH accurate data on the chloride content of the blood have been available longer than for the other blood constituents already described, its determination has not become a very common clinical procedure. This can hardly be ascribed to difficulties in the estimation of the chlorides, since it has been one of the simpler blood determinations, but must be attributed to either a lack of practical value in the test or to a nonappreciation of its significance, or possibly to both these factors.

Some of the observations recorded in the literature give the chloride content of the whole blood, others the content of the plasma or serum. Normally the chloride content of whole blood as sodium chloride amounts in round numbers to 0.45 to 0.50 per cent, while for the plasma the figures are about 0.12 per cent higher, i. e., 0.57 to 0.62 per cent. Since the plasma, rather than the whole blood, bathes the tissues of the body, it would seem more logical to study the chloride content of the plasma. Unfortunately, unless the plasma is quickly separated from the corpuscles there appears to be a gradual change (increase) in its chloride content owing to a passage of carbon dioxide from the plasma into the corpuscles (or its escape into the air) and of chlorides from the corpuscles to the plasma. In his paper dealing with the chlorides of blood plasma McLean[1] considered the influence of the plasma being allowed to stand in contact with the cells, but concluded that any change took place very slowly and that it was necessary only to centrifuge within two to three hours to avoid this danger. In their first paper on the plasma bicarbonate Van Slyke and Cullen[2] called attention to the effect which carbonic acid changes in whole blood might have on the chloride content of the plasma, a loss in bicarbonate resulting in an increase in the plasma chloride. This observation has recently been confirmed and extended by Fridericia,[3] who states that estimation of the chlorides of the plasma or serum from blood which has been kept in open receivers must give too high results, because chlorides have passed into the plasma (or serum) on account of the decreasing CO_2 tension. Similar observations regarding the increase in the plasma chlorides on standing have been made by Myers and Short.[4] This being the case, results obtained on whole blood would appear to be more trustworthy than those obtained on plasma. In any case a significant change in the blood chlorides should definitely affect the chloride content of whole blood as well as that of the plasma.

As far back as 1850 Carl Schmidt,[5] in his classic studies on the blood with special reference to cholera, gave figures for the chloride content of whole blood and plasma. Some of the observations recorded by Schmidt at that time have been recalculated to terms of sodium chloride and are given in Table I. The figures obtained in cholera appear to be low, while in the case of chronic edema with albuminuria there is a definite increase for both whole blood and plasma.

Table I

Observations of Schmidt on the Chloride Content of Whole Blood and Plasma Recalculated as NaCl

Case	Age	Sex	Chlorides as NaCl	
			Whole blood per cent	Plasma per cent
1. Normal	25	♂	0.437	0.589
2. "	20	♀	0.474	0.608
3. Cholera	26	♀	0.352	....
4. "	55	♂	0.431	....
5. "	20	♀	0.370	0.506
6. "	71	♂	0.367	0.492
7. "	23	♂	0.398	0.558
Diabetes	34	♂	0.461	0.572
4. Chronic edema with albuminuria	39	♂	0.552	0.646
5. Anasarca without albuminuria	42	♂	0.503	0.633

The French school was the first to emphasize the importance of the retention of chlorides in nephritis, particularly in those cases with edema, and to systematically employ restrictions of the chlorides in the diet in the treatment of these cases. The contributions of Widal and Javal[6, 7] are well known in this connection. These studies were later extended by Ambard,[8, 9, 10] who considered the relation of the chlorides of the serum to this general question, and constructed a formula[11] to express the rate of chloride excretion similar to his well-known formula for urea.

In this country McLean[1] has devoted considerable attention to this subject, working along lines somewhat similar to those of Ambard. In a fairly large series of normal individuals he found the plasma chloride to vary from 0.57 per cent to 0.62 per cent with a very constant chloride threshold of about 0.562 per cent. The threshold was calculated from the formula of Ambard and Weill[11] and confirms their observation on this point. McLean considered the question of the plasma chlorides in a number of pathologic conditions, the lowest observation being 0.50 per cent in a diabetic and the highest 0.84 per cent in a cardionephritic shortly before death. In general, relatively increased concentrations of chlorides were found in the plasma in certain forms of cardiac and renal disease, while decreased concentrations were noted in certain diabetic and fever patients, also after the action of digitalis, the decreased concentrations apparently resulting from a temporary or permanent lowering of the chloride threshold. Failure to excrete chlorides in pneumonia was found to be associated with a lowered concentration of chlorides in the plasma, excretion reappearing with a rise in the plasma chlorides. Edema was usually found to be accompanied by a relatively increased concentration of chlorides in the plasma, which ordinarily returned to the normal state with the disappearance of the edema.

In 1920 Allen[12] published a paper on arterial hypertension which has aroused considerable interest. He endeavored to show that salt retention (as shown by the plasma chloride) is the cause of pure hypertension, and also of the hypertension in many cases of kidney disease. This is a very fascinating explanation of hypertension, especially because restriction in the chloride intake affords such a simple means of treatment. Very recently

Mosenthal and Short[13] have carried out in this hospital studies on cases of so-called pure hypertension with special reference to the relation of the blood chlorides to the blood pressure. These experiments have not given support to Allen's theory. This problem has also been investigated by O'Hara and Walker[14] who point out that if salt is such a determining factor, one should expect to find the blood chlorides increased in cases of hypertension, and to observe a definite relation between the level of this substance and that of the blood pressure. According to their clinical observations, however, hypertension is compatible with normal blood chloride, normal tension is compatible with high blood chloride, and there is no relation between the height of the blood pressure and that of the blood chloride. They point out that the greatest salt retention in the body is observed in cases of nephritis with edema, and yet these usually show a comparatively low arterial tension.

In a very recent paper Allen and Sherrill[15] have described in detail their results on 180 severe cases of hypertension treated with close restriction of the sodium chloride intake, for periods of from one month to three years. Fully normal blood pressure was restored in only 19 per cent of the cases, but they believe that the relief of hypertension and other symptoms in 42 per cent of the remaining cases was sufficient to be regarded as a distinct therapeutic success. Complete failure, however, was encountered in 31 per cent of the cases. Allen and Sherrill conclude that, on the whole, the "pure" hypertension cases with high plasma chloride carry a better prognosis than those with low plasma chloride, perhaps because the pressure in the former group is governed more by a functional element of chloride retention and in the latter group more by strictly organic changes. The nephritic group shows by far the highest proportion of failures and deaths. According to Allen and Sherrill the chief requirement for success, which needs emphasis above everything else in the use of this treatment, is that sufficiently strict salt privation must be carried out for a sufficient length of time. In more than 60 per cent of their cases the best results could be obtained and maintained only by diets which kept the sodium chloride excretion below 0.5 gm. in each 24 hrs. They state that as symptoms of weakness and anorexia appear in some patients after several days to several weeks of salt privation, it is necessary to determine precisely the salt tolerance in each individual case so as to insure freedom from privation symptoms on the one hand and the best control of the hypertension on the other.

Höst[16] in a recent publication has presented a discussion of chloride metabolism with some interesting observations on several cases of acute nephritis. These cases showed edema, increased blood pressure, and a definite increase in the chloride concentration of the whole blood, figures ranging from 0.51 to 0.54 per cent. With improvement there was an increased chloride excretion and the blood chlorides returned to a normal concentration of 0.45 to 0.47 per cent.

From our experience it does not appear possible to draw the same definite conclusions regarding variations in the blood chlorides that may be made regarding other blood constituents discussed in the earlier chapters.

TABLE II

CHLORIDES OF WHOLE BLOOD IN NEPHRITIS

CASE	AGE	SEX	CHLORIDES AS NaCl	UREA N	SUGAR	PLASMA CO_2	CONDITION
			per cent	mg. to 100	per cent	c.c. to 100	
1. R. B.	50	♂	0.638	104	0.127	24	Chronic nephritis, died
2. S. B.	8	♂	0.625	89		25	Chronic diffuse nephritis (following nephrosis), uremia, marked edema, transfusion 2 days before last analysis, died
			0.625	86		20	
			0.588	109	0.104	21	
3. W. T.	35	♂	0.613	60	0.154	31	Cardio-nephritis, improved
			0.500	46	0.133	47	
4. W. N.	32	♂	0.600	32	0.158	23	Chronic diffuse nephritis, improved
			0.588	57	0.153	24	
			0.538	54		38	
5. W. B.	48	♂	0.600	16	0.150		Chronic myocarditis, improved
			0.463	21	0.176		
			0.463	12	0.117		
6. H. N.	65	♂	0.500	25	0.133	42	Chronic myocarditis, arteriosclerosis
			0.550	32	0.121	37	
			0.600	29	0.141		
			0.525	29		43	
			0.475	33		50	
7. S. T.	38	♂	0.594	100	0.136	20	Advanced nephritis, restricted chloride diet, died.
			0.538			20	
			0.513	161		20	
			0.480				
			0.394				
8. F. M.	37	♂	0.587	43	0.092		Cardio-nephritis
9. A. W.	8	♀	0.585	91	0.157	27	Acute nephritis
10. M. W.	44	♂	0.550	117	0.136		Diffuse nephritis, edema, died
11. M. D.	30	♂	0.537	43	0.121		Advanced nephritis
12. C. B.	48	♀	0.529	25	0.128		Cardiac decompensation, acute nephritis
13. M. I.	75	♂	0.494	121	0.172		Advanced nephritis
14. P. L.	49	♂	0.525	54	0.106	43	Chronic nephritis, uremia, died
			0.450	49	0.148	31	
			0.413	155	0.198	35	
15. B. L.	46	♀	0.413	49	0.197		Cardio-nephritis, died.
			0.425	72	0.174		
			0.450	40		60	
			0.413	74	0.241	55	
16. S. B.	42	♂	0.450	88	0.230	50	Chronic nephritis, uremia, died
			0.450	22	0.176		
			0.375	55	0.156	53	

An idea of the pathological variations which may occur in the blood chlorides, however, may be obtained from the data given in Tables II to V.

Table II presents data on the chlorides of whole blood in nephritis. In the larger number of cases of nephritis the figures for the blood chlorides are not especially abnormal, although both high and low figures may be encountered, the former being the most common. In cases of chronic interstitial nephritis the ability to eliminate chlorides does not appear to be impaired to nearly the same extent as the ability to eliminate nitrogen. With moderate dietary restrictions in chlorides the blood chlorides readily fall and may drop below normal, as in Case 7. The fall in the blood chlo-

rides in this case may be partly dependent upon another factor. A sudden rise in other crystalloids, in this instance urea, may result in a compensatory fall in the chlorides. As might be inferred from the data given in Table II, the tendency for chloride retention appears to be greater in cardionephritis than uncomplicated nephritis.

One renal condition which is associated with marked edema, but in which there does not appear to be an increase in the blood chlorides is nephrosis. This is quite contrary to what might be anticipated. Data illustrating this point are given in Table II of the preceding chapter.

TABLE III

CHLORIDES OF WHOLE BLOOD IN MISCELLANEOUS CONDITIONS

CASE	AGE	SEX	CHLORIDES AS NaCl	UREA N	SUGAR	PLASMA CO_2	CONDITION
			per cent	mg. to 100	per cent	c.c. to 100	
1. N. L.	24	♀	0.630	14	0.120		Eclampsia, marked edema
2. F. H.	27	♀	0.610	10	0.100	12	Eclampsia, edema, died
3. E. P.	20	♀	0.520	6	0.120	30	Eclampsia, slight edema
4. A. S.	24	♀	0.519	16	0.130	43	Postpartum eclampsia
5. S. R.	40	♂	0.575	12	0.120		Prostatic abscess, improved
6. F. O.	75	♂	0.561	32	0.105	51	Prostatic hypertrophy, operation, improved
			0.550	17	0.100		
7. C. P.	62	♂	0.563	19	0.140		Prostatic hypertrophy, operation, improved
8. R. K.	67	♂	0.456	28	0.142		Prostatic obstruction
9. J. Z.	23	♀	0.600	12	0.093		Pernicious anemia
10. J. D.	62	♂	0.600	16	0.133		Carcinoma of stomach, secondary anemia
11. J. S.	59	♂	0.563	49	0.127	56	Carcinoma of larynx, operation, improved
12. T. M.	58	♂	0.350	91	0.125	52	Carcinoma of tongue, died.
13. W. I.	57	♂	0.400	111		12	Carcinoma of kidney, died
14. M. F.	30	♂	0.325	76	0.190	76	Perforated appendix, died
15. N. M.	52	♀	0.350	19	0.093	81	Acute perforated cholecystitis, acute pancreatitis, 40 gm. $HNaCO_3$, improved
			0.400	12		70	
			0.438	14	0.136		
16. E. L.	43	♀	0.375	42	0.294		Bronchopneumonia, died
17. J. H.	62	♂	0.519				Essential hypertension
			0.457	14			
			0.443				
18. A. K.	40	♀	0.500	12	0.134		Essential hypertension
19. J. M.	45	♂	0.492	13	0.121		Essential hypertension
20. G. M.	49	♂	0.432	16	0.101		Essential hypertension
21. E. K.	64	♀	0.478		0.390	44	Diabetes mellitus
22. C. J.	50	♀	0.444		0.375		Diabetes mellitus
23. J. J.	40	♂	0.444		0.440	42	Diabetes mellitus
24. D. C.	43	♂	0.425	15	0.348	39	Diabetes mellitus

Blood chloride findings in miscellaneous conditions are presented in Table III. In eclampsia there appears to be a fairly definite relationship between the severity of any edema and the height of the blood chlorides. Edema is generally present when the chlorides much exceed 0.50 per cent. An increase in the blood chlorides is quite common in eclampsia. Moderate chloride retention is not infrequently encountered in cases of prostatic obstruction. Whole blood chlorides are quite generally increased in severe anemia, probably for the reason that the proportion of plasma here is much

greater than in normal blood. As already pointed out a sudden increase in any of the blood crystalloids appears to be followed by a compensatory drop in the chlorides. This is well brought out by the data on Cases 14 and 15, where a rise in the blood bicarbonate was accompanied by a marked fall in the chlorides. Pyloric obstruction with vomiting is likewise accompanied by a fall in the blood chlorides. The loss of hydrochloric acid has been advanced by several workers as an explanation of this drop in the blood chlorides, but since the condition is accompanied by an increase in the blood bicarbonate, the author believes the low chlorides find the same explanation as in the two cases referred to above. In cases of severe diabetes the chlorides are frequently low, possibly for a similar reason, the crystalloid increased in this case being the sugar. As will be noted in the

TABLE IV

BLOOD CHLORIDES IN A CASE OF BRONCHOPNEUMONIA*
L. B., female, aged 38

DATE 1921	WHOLE BLOOD CHLORIDES AS NaCl	NON-PROTEIN N	UREA N	URIC ACID	CREATININE	SUGAR	CO_2 COMBINING POWER	REMARKS
		MG. TO 100 C.C.						
	per cent					per cent	c.c. to 100	
11/14	0.325	40	19	3.4	2.6	0.13	52	Tempt. 104° F., extensive consolidation 12 hrs. after beginning of crisis
11/16	0.400	53	21	6.6			66	
11/20	0.450	94	23	6.8	2.5			Tempt. normal, lungs clear, toxic
11/28	0.538	57	20	3.3	2.4	0.13		Lungs clear, improved
12/ 4	0.515	26	10	3.2	2.1	0.10		Lungs clear, recovered

*Unpublished observations of J. A. Killian.

TABLE V

BLOOD CHLORIDES IN A CASE OF BICHLORIDE POISONING*

DATE 1921	WHOLE BLOOD CHLORIDES AS NaCl	URIC ACID	UREA N	CREATININE	CO_2 COMBINING POWER
	per cent	mg. to 100	mg. to 100	mg. to 100	c.c. to 100
5/ 9	0.495		22		36
5/11	0.338	8.3	90	10.7	
5/13	0.388		88	9.1	30
5/15	0.114		98	12.0	27
5/17	0.207		73	12.0	
5/19	0.250	8.0	75	9.3	
5/21	0.363	2.8	91	7.8	81
5/27	0.500	1.0	18	4.4	61
6/ 6	0.525	1.9	13	2.2	

R. S., female, aged 19, took 15 grains $HgCl_2$. Admitted to hospital evening of May 7. Four to five liters of alkaline fluid introduced as gastric lavage, colonic irrigations and enemas and hot packs given May 7 to 27. In addition to this, 1000 c.c. of 0.9 per cent NaCl were introduced intravenously daily from May 16 to 25. No anuria and no edema. Clinical improvement noted about May 20. Discharged from hospital cured June 19.

*Data taken from Killian.[17]

table the blood chlorides are practically normal in the four cases of essential hypertension, for which data are given.

It has long been recognized that the chloride excretion is much diminished in severe pneumonia and this was at one time thought to result from impaired renal function, but the advent of blood analyses has shown this to be due to a decreased concentration of the blood chlorides. A rise in the blood chlorides from 0.325 per cent to normal in a case of severe pneumonia following recovery is nicely shown in Table IV.

That the blood chlorides may occasionally reach an unusually low figure is evident from the data given in Table V in a case of bichloride poisoning. This fall in the chlorides was accompanied by a corresponding rise in the nitrogenous constituents.

General Discussion

Although the practical value to be derived from the estimation of the blood chlorides can hardly be compared with that of some of the other blood constituents already described, still it is believed that the preliminary estimation of the chloride content of the blood in cases of nephritis may often be of great assistance, particularly in indicating the extent to which chlorides should be restricted in the diet. Furthermore, this estimation should be utilized to determine when the blood chlorides have returned to their normal level. It is believed that in the past, chloride restrictions have often been made when they were not indicated, and, when indicated, have been continued until in some cases the chlorides of the blood reached a subnormal concentration.

In general it may be stated that high blood chlorides have been found in nephritis, certain cardiac conditions, in eclampsia, prostatic obstruction, in anemia and some cases of malignancy (possibly due to an accompanying renal involvement), while low values have been observed notably in fevers, diabetes and pneumonia. The chloride retention in most cases of nephritis apparently results from impaired renal function.

The excretion of chlorides and nitrogen seem to be fairly independent functions. The function of excreting chlorides in interstitial nephritis appears to be much less impaired than that of excreting nitrogen. Consequently a restriction in the chloride intake may fairly quickly restore the chlorides to normal. Cases are occasionally encountered, however, with only slight nitrogen, but with considerable chloride retention. With this type of condition dietary restrictions in the chlorides result in marked clinical improvement.

When cases of advanced nephritis with marked nitrogen retention are put on a restricted chloride diet it is sometimes noted that the blood chlorides drop to a subnormal level. Such subnormal figures are occasionally found in severe diabetes. A possible explanation for this is that, owing to the large amounts of urea and sugar present in the blood in these conditions, less chloride is needed to maintain normal osmotic conditions. Likewise with a sudden rise in the blood bicarbonate, there may be a compensatory fall in the chlorides.

The maintenance of the normal osmotic relations of the blood would appear to depend in large part upon the blood chlorides, since they are apparently able to compensate for abnormal variations in other crystalloids.

High figures for whole blood chlorides in anemia are probably dependent upon the larger proportion of plasma in the sample analyzed.

It is of considerable interest that the chloride retention in pneumonia is associated with a decrease in the chloride concentration of the blood.

Estimation of the Blood Chlorides

The estimation of the chloride content of blood is made with the aid of volumetric methods long employed in analytical chemistry. It has simply been necessary for the physiologic chemist to completely remove the blood proteins so that the chlorides could be titrated. When care is employed, it is possible to ash the blood and determine the chlorides in the ash, but such a method is not suited to practical purposes. A number of different methods have been suggested for the precipitation of the proteins, but none of these has worked as well in our hands as the use of picric acid first employed by Van Slyke and Donleavy[18] for this purpose. Since picric acid is used for the precipitation of the proteins in the methods for creatinine and sugar estimation already described, it is possible to save considerable time by utilizing a portion of this same filtrate. (Myers and Short[4] have shown that this 1 to 5 dilution of the blood extracts the chlorides quite as well as the 1 to 20 dilution employed by Austin and Van Slyke.[19]) In going over the various methods of chloride estimation Greenwald and Gross[20] have pointed out that a purine-silver picrate is formed as a result of the addition of silver nitrate to the picric acid filtrate and that this leads to an average plus error of about 3 per cent on whole blood, although on plasma the error is probably not as great. They are inclined to favor the estimation of the chlorides on the Folin-Wu tungstic acid filtrate as suggested by Rieger[21] and Whitehorn[22] (See page 157). Myers and Short originally attempted the estimation of chlorides on this tungstic acid filtrate, but abandoned this precipitation in favor of the picric acid filtrate, because they could obtain better duplicates and better recoveries of added sodium chloride.

Having obtained a blood filtrate suitable for the chloride estimation, one is confronted with the selection of a method of chloride titration. McLean and Van Slyke[23] have suggested an iodometric method which is delicate and gives a sharp end point when the starch solution is fresh. We are inclined to prefer, however, the well known thiocyanate titration of Volhard, using iron as an indicator. The end point in the titration is possibly not as sharp, but the solutions are permanent, and may be readily prepared by diluting the solutions employed in the Volhard-Harvey[24] method for urine.

Method.[4]—The chloride titration may be carried out on the same picric acid filtrate as employed for the estimation of the creatinine and the sugar already described, or the following technic may be employed for the precipitation of the proteins: To 12 c.c. of distilled water in a 20-25 c.c. centrifuge tube are added 3 c.c. of whole blood (or plasma), and then about 0.5 gm. of dry picric acid. The mixture is now stirred until protein precipita-

tion is complete and the mixture turns a bright yellow color. The precipitate is next thrown down in the centrifuge, and the supernatant fluid filtered into a dry tube.

Five c.c. of the filtrate are then pipetted into a centrifuge tube of 25 c.c. capacity and 20 c.c. of the standard silver nitrate-acidified ferric alum indicator solution* added. The contents are stirred to insure thorough mixing and the silver chloride precipitate thrown down in the centrifuge. The clear supernatant fluid is decanted into a clean dry beaker and 20 c.c. pipetted into a small porcelain evaporating dish for titration.

The titration is made with ammonium thiocyanate solution† of such strength that 1 c.c. is the equivalent of 1 c.c. of the silver indicator solution. The end point is definite and consists of the first permanent tinge of reddish brown which extends throughout the mixture. Some experience may be necessary before the end point is always recognized, but thereafter there need be no difficulty in obtaining exact duplicate titrations. Passing the end point by one drop will introduce an error ordinarily of about 0.5 per cent in estimating chlorides in 100 c.c. of blood.

The calculation may be carried out with the aid of the following formula:

$$20 - (\text{titer} \times \tfrac{5}{4}) \times 0.5 \times 100 = \text{mg. of sodium chloride in 100 c.c. of whole blood or plasma.}$$

The 5 c.c. of picric acid filtrate contains the chlorides from 1 c.c. of blood. Since only four-fifths of this is titrated, it is necessary to introduce a correction in the formula. The 20 is the amount of standard silver solution employed, 0.5 the equivalent strength in mg. of NaCl and the 100 the factor required to convert the figures to mg. per 100 c.c.. If the figures are desired in per cent, this may obviously be obtained by moving the decimal point forward three places.

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†The ammonium thiocyanate solution is standardized against the silver nitrate and made of equivalent strength. It contains approximately 0.65 gm. of the thiocyanate to 1000 c.c. It is one-twentieth of the strength employed for the Volhard-Harvey method in urine.

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CHAPTER IX

FOLIN AND WU SYSTEM OF BLOOD ANALYSIS

THE system introduced by Folin and Wu[1] for the estimation of certain nonprotein constituents in the blood depends primarily upon the use of the same protein free filtrate for the several determinations. With the supplements so far added to their system the following determinations may be made upon the protein free filtrate: nonprotein nitrogen, urea, creatinine, creatine, uric acid,[5] amino acid nitrogen,[4] sugar[2] and chlorides.[3] As their protein precipitant Folin and Wu introduced the use of tungstic acid, which is unquestionably better adapted to all these different determinations, than any other precipitant so far proposed. The tungstic acid is prepared at the time of the protein precipitation, by the addition to the blood, already diluted with 7 volumes of water, of 1 volume of 10 per cent sodium tungstate, followed by 1 volume of $\frac{2}{3}$ N sulfuric acid.

Folin and Wu's object in introducing their so-called system of blood analysis was to combine a number of different analytical procedures into a compact system, the starting point for which should be a protein-free filtrate suitable for the largest possible number of different determinations. Where it is necessary to carry out a number of different analyses upon a protein-free filtrate, it is an obvious economy of time and material to be able to utilize the same filtrate. With this procedure Folin and Wu found it possible to effect a considerable economy in the use of blood. Previous to the simplification of their uric acid method, they stated that the nonprotein nitrogen, urea, creatinine, creatine, uric acid and sugar might all be determined in the filtrate obtained from 10 c.c. of blood. With the further economy effected by the simplification of the uric acid method, the chlorides or amino acid nitrogen can be included in the determinations which may be carried out on the above filtrate.

There are conditions under which the use of the so-called system may hardly be considered an advantage. With a small amount of material it is not always possible to decide in advance how that can best be utilized. One would proceed differently with a high urea than with a high sugar, and in either case the CO_2-combining power should be estimated. Obviously if the sugar were to be done, the Folin-Wu method might be used, but if the urea should be the particular estimation indicated, the preliminary precipitation of the protein is of questionable advantage. According to the very satisfactory Van Slyke-Cullen modification of the Marshall urease method the ammonia is aerated directly from the blood after the digestion and then titrated, or in the Myers' modification nesslerized (see Chapter II). Although tungstic acid is an excellent protein precipitant for the nonprotein nitrogen estimation, according to Hiller and Van Slyke[7] it does bring down

more peptid nitrogen than trichloracetic acid. In conditions, such for example as eclampsia, pneumonia and intestinal obstruction, where there is special interest in the peptid nitrogen, trichloracetic acid is probably to be preferred as the protein precipitant. For the estimation of uric acid and amino acids, tungstic acid is most satisfactory, and it likewise serves excellently in the determination of sugar and creatine. In the case of the creatinine estimation, the use of the tungstic acid necessitates a greater dilution of the blood (1-15), than with the original method (1-5) where picric acid is used as the protein precipitant. With nephritic bloods high in creatinine, the greater dilution is an advantage, but with normal bloods, the color development is so weak that it is difficult to read satisfactorily. According to the observations of Rieger, and Whitehorn,[3] the tungstic acid filtrate serves well for the estimation of chlorides. However, Myers and Short, who apparently were the first to attempt the use of this filtrate for the chloride estimation, were unable to obtain as good duplicates or as good recoveries of added chloride as when picric acid was employed as the protein precipitant.

Collection of Blood

The blood may be drawn, as already described (page 29) directly into a bottle containing the anticoagulant or first into a large glass syringe and then immediately transferred to the bottle. In either case the blood should be mixed at once with the anticoagulant by giving the bottle a gentle rotary motion. (It is inconvenient to gum up the stopper by shaking.)

As has long been recognized, calcium is necessary for the coagulation of blood. Since oxalates are the best calcium precipitants, they are generally used as the anticoagulant. In the Folin-Wu system an excess of oxalate interferes with the tungstic acid precipitation of the protein, while the potassium in potassium oxalate tends to give insoluble precipitates with the uric acid reagent. Potassium oxalate has generally been used because of its great solubility. It is about seven times as soluble as the sodium salt. *About 20 mg. of potassium oxalate are ample for 10 c.c. of blood.* Two drops of a 20 per cent solution contain rather more than 20 mg.

Recently Folin[6] has recommended the use of lithium oxalate,* which has a fair solubility (about 6 per cent). One mg. per c.c. of blood is abundant for the prevention of coagulation. This may be conveniently used in the form of oxalate cloth.†

*Since lithium oxalate is apparently unavailable in the market, Folin[6] suggests the following method of preparation: To 50 gm. of lithium carbonate in a liter beaker add 85 gm. of crystallized oxalic acid. Pour on the mixture about 1 liter of hot water (70° C.). Stir cautiously, to avoid loss of liquid by foaming. Evaporate the resulting solution to dryness, and powder.

†To prepare the oxalate cloth, cut 80 gm. bird's-eye cotton cloth, free from starch, into strips 10 cm. by 40 to 50 cm. Transfer 10 gm. of lithium carbonate and 17 gm. of oxalic acid to a liter beaker. Add 240 c.c. of water (70° C.) and shake. Transfer the solution to a plate. Draw the cotton strips through it, and hang up to dry. The cloth when dry will contain about 20 per cent of lithium oxalate, and a very small piece (50 mg.) is adequate for 15 or even 20 c.c. of blood. Since the lithium oxalate solution is practically saturated, the cloth may be given an additional charge of oxalate by repeating the above process, if desired.

Precipitation of Proteins

The precipitation of the blood proteins by means of Folin and Wu's tungstic acid reagent is made as follows:[1, 6]

Method.—Pipette a measured quantity (5 to 15 c.c.) of oxalated blood into an Erlenmeyer flask having a capacity of fifteen to twenty times that of the volume taken. For this purpose Folin's special blood pipette may be used. Dilute the blood with 7 volumes

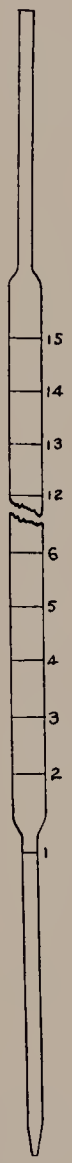

Fig. 16.—Folin's blood pipette.*

of water and mix to lake. Add (Folin's blood pipette serves well) one volume of a 10 per cent solution of sodium tungstate† and mix. With another pipette add slowly to the

*Dr. Folin states that the blood pipettes and other special glassware used in the system were made for him by the Emil Greiner Co., 55 Fulton Street, New York.

†*Sodium Tungstate.*—A 10 per cent solution is employed. Not all brands of sodium tungstate are suitable. The sodium tungstate must be very easily soluble in cold water. If it is not easily soluble, its solutions are not alkaline to phenolphthalein, and this shows that the product consists of complex tungstates (paratungstates). Folin[6] states that such tungstates can be rendered serviceable as follows: Prepare a hot 10 per cent solution. Allow it to cool. Titrate 25 c.c. of the solution with 10 per cent sodium hydroxide, using phenolphthalein as indicator. The right end point is obtained only when the pink color produced by the alkali remains for at least 3 minutes. The titration figure so obtained will serve as a basis for calculating how much sodium hydroxide is required for 100 gm. of sodium tungstate

contents of the flask (with shaking) one volume of ⅔ normal sulfuric acid.* Close the mouth of the flask with a rubber stopper and shake. If the conditions are right, hardly a bubble of air will form as a result of the shaking. After standing for about 5 minutes the color of the coagulum should change from bright red to dark brown. If this change in color does not take place, the coagulation is incomplete, usually because too much oxalate is present. In such a case the sample can generally be saved by adding 10 per cent sulfuric acid, one drop at a time, shaking vigorously after each drop, and continuing until there is practically no foaming and until the dark brown color has set in.

Pour the mixture on a filter large enough to hold the entire contents of the flask and cover with a watch glass. If the filtration is begun by pouring the first few c.c. of the mixture down the double portion of the filter paper and withholding the remainder till the whole filter has been wet, the filtrates are generally as clear as water from the first drop. If a filtrate is not perfectly clear, the first 2 or 3 c.c. may have to be returned to the funnel.

The preparation of protein-free blood filtrates by this process is so simple that no one need go astray, provided that the sodium tungstate and the ⅔ normal sulfuric acid are correct. The only doubtful point is the quality of the sodium tungstate used. Tests for the purity of the tungstate are given in the footnote below. The acid is intended to set free the whole of the tungstic acid with about 10 per cent excess (and to neutralize the carbonate usually present in commercial tungstates). A great excess of sulfuric acid must not be used, for if this is the case a large part of the uric acid will be lost. The blood filtrates should be only slightly acid to congo red.

The protein blood filtrates are not acid enough to prevent bacterial decomposition. If the filtrates are to be kept for any length of time, more than two days, some preservative, such as a few drops of toluene or xylene, should be added.

Precipitation of Proteins from Plasma or Serum

For some purposes blood plasma or blood serum may give more instructive analytical data than are obtained from whole blood (see paper by Berglund[8]). The use of plasma or serum, however, is not as economical in point of blood required.

An idea of the difference between the concentration of the several nitrogenous waste products in whole blood and plasma may be gained from an inspection of Table I, taken from data of Berglund.[8] The findings in the case of uric acid are especially noteworthy. The cell membranes of the corpuscles are not ordinarily very permeable to uric acid, and consequently

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in order to convert the paratungstate into the true simple tungstate. Add the calculated amount, and heat until solution is obtained.

For large scale work it is advantageous to prepare two liters or more of 20 per cent. sodium tungstate solution which is weakly alkaline to phenolphthalein. From this stock solution the calcium will gradually settle out, and the clear supernatant solution can then be used as desired for the preparation of the Folin-Denis uric acid reagent or (after diluting with an equal volume of water) as the blood protein precipitant.

**Sulfuric Acid.*—A ⅔ normal sulfuric acid solution is employed. This may be prepared by adding 35 gm. C. P. sulfuric acid to about 700 c.c of water in a liter volumetric flask, cooling, then making up to volume. Although this solution will usually be found correct, it is necessary to check it up by titration. The ⅔ normal sulfuric acid is intended to be equivalent to the sodium content of the tungstate, so that when equal volumes are mixed, practically all the tungstic acid is set free without the presence of an excess of sulfuric acid. The tungstic acid set free is nearly all taken up by the proteins, and the blood filtrates obtained are therefore only slightly acid to congo red paper.

most of the uric acid is generally found in the plasma. For this reason the uric acid content of the plasma may be much higher than that of the whole blood.

TABLE I

COMPARATIVE RETENTION OF THE SEVERAL NITROGENOUS WASTE PRODUCTS
(After Berglund[8])

	CASE	AMINO-ACID N	UREA N	CREATININE	URIC ACID	UNDETERMINED RESIDUAL N	TOTAL NON-PROTEIN N	CORPUSCLES VOLUME
		MG. PER 100 C.C.						PER CENT
Whole Blood	1	5.0	13	1.8	3.7	19	39	45
	2	6.4	49	2.0	4.5	7.4	65	50
	3	4.5	49	3.0	4.7	22	78	27
	4	5.8	60	7.7	4.8	37	107	23
	5	7.6	91	7.2	6.4	41	144	25
	6	8.4	174	12.9	13.6	75	267	39
	7	7.3	193	16.0	12.0	48	258	42
Plasma	1	4.3	13	1.8	3.7	20	39	
	2	5.8	47	2.3	6.4	18	74	
	3	3.5	52	3.0	6.1	18	77	
	4	5.4	71	8.1	6.8	21	103	
	5	6.2	109	8.1	9.3	23	144	
	6	7.5	214	14.5	18.4	52	285	
	7	7.3	234	19.2	21.0	51	306	
Corpuscles	1	5.9	13	1.8	3.7	18	39	
	2	7.2	52	1.5	1.6	..	51	
	3	7.4	41	3.0	0.9	31	81	
	4	7.2	26	6.5	0.0	86	122	
	5	11.8	38	4.4	0.0	93	144	
	6	9.7	113	10.5	6.1	109	238	
	7	7.3	138	11.7	0.0	43	193	

Where plasma or serum is precipitated, it is only necessary to use one-half volume each of the sodium tungstate and ⅔ normal sulfuric acid, and furthermore by reducing the preliminary dilution one may obtain a more concentrated filtrate than in the case of whole blood. For example, 5 c.c. of plasma, 25 c.c. of water, 2.5 c.c. of sodium tungstate and 2.5 c.c. of ⅔ normal sulfuric acid give a total volume of 35 c.c. and a final dilution of 1 to 7, instead of the 1 to 10 employed for whole blood.

Estimation of Nonprotein Nitrogen

Method.—The Kjeldahl digestion is most conveniently made in Pyrex ignition test tubes (200 x 25 mm.) which have been graduated at 35 c.c. and at 50 c.c. Pipette 5 c.c. of the blood filtrate into such a test tube. The test tube should either be dry or rinsed with alcohol to reduce the danger of bumping. Add 1 c.c. of the sulfuric-phosphoric acid digestion mixture* and a quartz pebble. Boil vigorously over a micro-burner until the characteristic dense acid fumes begin to fill the tube. This will occur in from 3 to 7 minutes depending on the size of the flame. When the test tube is nearly full of fumes,

**Folin's Acid Digestion Mixture.*—Mix 300 c.c. of phosphoric acid syrup (85 per cent) with 100 c.c. of concentrated sulfuric acid. Transfer to a tall cylinder, cover well to exclude the absorption of ammonia, and set aside for sedimentation of calcium sulfate. This sedimentation is very slow, but in the course of a week or so the top part is clear and 50 to 100 c.c. can be removed by means of a pipette. (It is not absolutely necessary that the calcium should be thus removed, but it is probably a little safer to have it done.) To 100 c.c. of the clear acid add 10 c.c. of 6 per cent copper sulfate solution and 100 c.c. of water. One c.c. of this solution is employed for the digestion.

reduce the flame sharply so that the speed of the boiling is reduced almost to the vanishing point. Cover the mouth of the test tube with a watch glass. Continue the heating very gently for 2 minutes, counting from the time the test tube became filled with fumes. If the oxidations are not visibly finished at the end of two minutes, the heating must be continued until the solution is nearly colorless. Usually the solution becomes colorless at the end of 20 to 40 seconds. At the end of 2 minutes remove the flame and allow the digestion mixture to cool for 70 to 90 seconds. Then add 15 to 25 c.c. of water. Cool further, approximately to room temperature, and add water to the 35 c.c. mark. Add, preferably with a pipette, 15 c.c. of dilute Nessler's solution.* Insert a clean rubber stopper and mix. If the solution is turbid, centrifuge a portion before making the color comparison with the standard. The standard most commonly required is 0.3 mg. of N† (in the form of ammonium sulfate) in a 100 c.c. volumetric flask. Add to it 2 c.c. of the sulfuric-phosphoric acid mixture, about 50 c.c. of water, and 30 c.c. of Nessler solution. Fill to the mark and mix. The unknown and the standard should be nesslerized at approximately the same time.

Calculation.—The various factors which need to be considered in calculating the results in mg. of nonprotein N per 100 c.c. of blood are given in the following formula: $\frac{20}{R} \times 0.3 \times \frac{50}{100} \times \frac{100}{0.5}$, in which 20 stands for the depth of the standard in mm., R the reading of the unknown, 0.3 the mg. N in the standard, 50 the dilution of the unknown, 100 the dilution of the standard, 100 the factor to convert to 100 c.c. of blood and 0.5 the equivalent of the amount of blood employed. When simplified this formula gives: 20 divided by the reading of the unknown, times 30 gives the nonprotein nitrogen in mg. per 100 c.c. of blood.

Folin points out that many seem to have trouble in obtaining perfectly clear solutions when nesslerizing the digestion mixtures obtained with blood filtrates. The cause is lack of suitable alkalinity in the Nessler solution. This may be ascertained as follows:

Twenty c.c. of normal hydrochloric acid may be titrated with the Nessler solution, and if the solution is substantially correct, a good end point will be obtained at 11 to 11.5 c.c. with phenolphthalein as indicator. If an end point is obtained much below 11 c.c., as at 9.5 c.c., the Nessler solution is too alkaline and turbidity is likely to occur.

Turbidity due to excess of alkalinity may likewise be produced because the sulfuric-phosphoric acid mixture is too weak. If 5 c.c. of the dilute acid

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**Folin's Modified Nessler Reagent.*—This reagent is essentially a solution of the double iodide of mercury and potassium (HgI_2, 2KI) containing sodium or potassium hydroxide. Folin's method of preparing the double iodide is as follows: Transfer 150 gm. of potassium iodide and 110 gm. of iodine to a 500 c.c. Florence flask; add 100 c.c. of water and an excess of metallic mercury, 140 to 150 gm. Shake the flask continuously and vigorously for 7 to 15 minutes, or until the dissolved iodine has nearly all disappeared. The solution becomes hot. When the red iodine solution has begun to become visibly pale, though still red, cool in running water, and continue the shaking until the reddish color of the iodine has been replaced by the greenish color of the double iodide. This whole operation usually does not take more than 15 minutes. Now separate the solution from the surplus mercury by decantation and washing with liberal quantities of distilled water. Dilute the solution and washings to a volume of two liters. If the cooling were begun in time, the resulting reagent is clear enough for immediate dilution with 10 per cent alkali and water, and the finished solution can at once be used for nesslerizations.

Preparation of the double iodide as described above is not only less expensive than when mercuric iodide is employed, but it also avoids the impurities often found in the latter.

From a completely saturated sodium hydroxide solution, containing about 55 gm. of NaOH per 100 c.c., decant the clear supernatant liquid and dilute to a concentration of 10 per cent. (It is desirable to ascertain by titration that a 10 per cent solution has been obtained with an error of not over 5 per cent.) Introduce into a large bottle 3,500 c.c. of 10 per cent sodium hydroxide solution, add 750 c.c. of the double iodide solution, and 750 c.c. of distilled water, giving 5 liters of Nessler's solution.

In the absence of modifying circumstances, such as the presence of much acid or alkali, this reagent should be added in the proportion of 10 c.c. per 100 c.c. of the volume to which the nesslerized solution is to be diluted. As a general rule the graduated flask, cylinder or test tube should be at least two-thirds full before adding the Nessler reagent. If attention is not given to this detail, turbid mixtures are obtained and turbid solutions must never be used for color comparisons.

†A nitrogen standard containing 0.3 mg. N per 5 c.c. may be prepared by dissolving 0.283 gm. pure ammonium sulfate in water and making up to 1 liter.

(1:1) are further diluted 10 times (to 50 c.c.), 10 c.c. of the solution so obtained, when titrated with the Nessler solution and phenolphthalein as indicator, should give a fairly good end point at 9 c.c. to 9.3 c.c.

Estimation of Urea

Method.[1, 6]—Pipette 5 c.c. of the tungstic acid blood filtrate into a Pyrex ignition tube (200 x 25 mm.). This test tube must be rinsed with nitric acid and then with water if it has contained Nessler's solution, since a trace of mercury compounds is very injurious to urease. Add 2 drops of the buffer mixture* and then introduce 1 c.c. of urease solution† or half a square inch of urease paper.†† Immerse the test tube in warm water

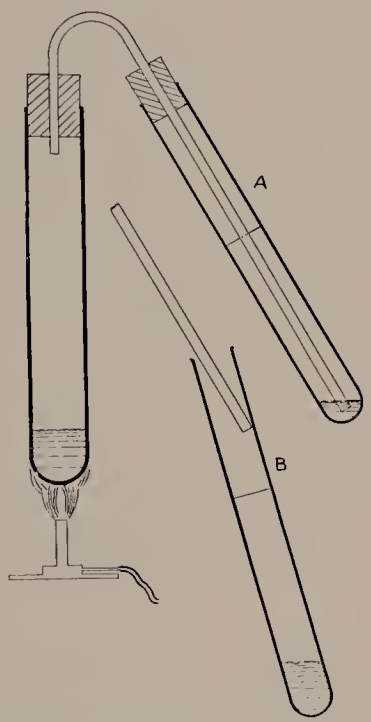

Fig 17.—Folin's arrangement for distilling ammonia in urea estimation (Jour. Biol. Chem., 1919, xxxviii, 96.) *A*, at beginning; *B*, towards end of distillation.

*Folin suggests two buffer mixtures for urease decomposition. To preserve a substantially neutral reaction during the decomposition of urea by means of urease, mixtures of mono- and disodium phosphates in the proportion of 1 molecule of the former to 2 of the latter, and in molar concentration, are usually employed.

(a) Dissolve 69 gm. of monosodium phosphate and 179 gm. crystallized disodium phosphate in 800 c.c. of warm distilled water. Cool and dilute to a volume of 1 liter. Preserve with 1 to 2 c.c. of toluene.

Folin questions whether the maintenance of neutrality is adequate to explain fully the accelerating action of phosphates on the urea decomposition, because pyro- and metaphosphates seem to be more effective than orthophosphates. An excellent buffer mixture may be obtained as follows:

(b) Dissolve 14 gm. of sodium pyrophosphate ($Na_4P_2O_7$, 10 H_2O) in enough half normal phosphoric acid to make a volume of 100 c.c. The half normal phosphoric acid is made by diluting 20 c.c. of 85 per cent phosphoric acid to 1 liter and titrating 5 c.c. with tenth normal alkali, and phenolphthalein as indicator, to a faint pink color. On the basis of titration, dilute the acid to a substantially correct half normal solution. The pyrophosphate-phosphoric acid mixture gives a faint color with rosolic acid. Five c.c. when titrated with tenth normal alkali and phenolphthalein should give a faint but distinct color with about 18 c.c. of the alkali.

†*Urease Extract (Folin).*—Wash about 3 gm. of permutit in a flask once with 2 per cent acetic acid, then twice with water; add 5 gm. of fine jack bean meal (may be obtained from Arlington Chemical Co.) and 100 c.c. of 15 per cent alcohol (16 c.c. of ordinary alcohol plus 84 c.c. of water). Shake gently but continuously for 10 to 15 minutes, pour on a large filter and cover with a watch glass. The filtrate contains practically the whole of the urease and extremely little of other materials. The urease solution will keep for about a week at room temperature and for 4 to 6 weeks in an ice box.

††*Urease Paper (Folin).*—Shake 30 gm. of jack bean powder with 10 gm. of permutit and 20 c.c. of 16 per cent alcohol for 10 to 15 minutes. Pour on one or two filters, and as soon as the filtration is substantially finished, pour the filtrate into a clean flat-bottomed dish. Draw strips of filter paper through the solution and hang them up to dry. They dry very quickly, and, once dry, the urease seems to keep just as well as it does in the original jack bean powder. Half a square inch of such a paper is enough for each blood urea determination.

at 40 to 55° C., and incubate for 5 minutes or let it stand at room temperature for 15 minutes and as much longer as is convenient.

The ammonia formed can be conveniently and quickly distilled into 2 c.c. of 0.05 normal hydrochloric acid contained in a second test tube. The second test tube should not be so heavy as the ordinary test tubes and should be graduated at 25 c.c. A simple and compact arrangement for this distillation is indicated in Fig. 17. The test tube which serves as a receiver is held in place by means of a rubber stopper in the side of which has been cut a fairly deep notch to permit the escape of air (and some steam). The delivery tube must be so adjusted as to reach below the surface of the hydrochloric acid solution in the receiver before distillation is begun. Add to the hydrolyzed filtrate a dry pebble, 2 c.c. of saturated borax solution, and a drop or two of paraffin oil; insert firmly the rubber stopper carrying both delivery tube and receiver, and boil moderately fast over a microburner for 4 minutes. The size of the flame should never be cut down during distillation, nor should the boiling be so brisk that the emission of steam from the receiving tube begins before the end of 3 minutes. At the end of 4 minutes slip off the receiver from the rubber stopper and put it in the position shown in the illustration above. Continue the distillation for 1 minute more and rinse off the lower outside part of the delivery tube with a little water. Cool the distillate with running water, dilute to about 20 c.c., and add 2.5 c.c. of Nessler's solution (described in footnote, page 148). Fill to the 25 c.c. mark and compare in the colorimeter with a standard containing 0.3 mg. of N in a 100 c.c. volumetric flask which has been nesslerized with 10 c.c. of Nessler's solution. The standard and unknown should always be nesslerized as nearly simultaneously as practicable.

Calculation.—The principles of the calculation are the same as for the nonprotein nitrogen estimation (see page 148). However, to obtain the mg. urea nitrogen per 100 c.c. of blood it is simply necessary to multiply 20 (the height of the standard in mm.) by 15 and divide by the colorimetric reading. It should be borne in mind that the standard containing 0.3 mg. of N is diluted to 100 c.c., while the unknown which corresponds to 0.5 c.c. of blood, is diluted to only 25 c.c. (in the case of normal blood).

Urea Estimation by Means of the Autoclave

When a large number of urea determinations are to be made or when creatine determinations are also made, it is sometimes convenient to decompose the urea of the blood filtrate by heating under pressure.

Method.—To 5 c.c. of the blood filtrate in a 75 c.c. test tube is added 1 c.c. of normal acid; the mouth of the test tube is covered with tinfoil, and the test tube with contents is then heated in the autoclave at 150° C. for 10 minutes. Allow the autoclave to cool to below 100° C. before opening. The ammonia is then distilled off exactly as in the first process described except that 2 c.c. of 10 per cent sodium carbonate are substituted for the borax, or it may be removed by aeration in the usual manner.

Folin and Wu[2] state that they are not prepared to say that the autoclave process does not give results that are slightly high, but they point out whether one finds 15 instead of 14 mg. of urea nitrogen is of comparatively small consequence. The chief merit of the autoclave process for decomposing urea in blood filtrates lies perhaps in the fact that by its help one is sure to get all the urea nitrogen; the value may be too high, but not too low. According to the observations of Folin and Wu the results obtained by the autoclave process are as a matter of fact usually identical and rarely as much as 1 mg. per 100 c.c. of blood higher than those obtained by the urease process.

Urea Estimation by Aeration

The removal of the ammonia formed from the blood urea by urease, or by heating under pressure, may be driven into the receiver by an air current plus an alkali, instead of by the distillation process described above. The aeration process gives perfectly reliable results, if a good air current is available. The only precaution which experienced investigators are likely to overlook is that the rubber tubing used for connections needs to be rinsed with water before being used the first time, and, later also, if the tubing has been idle for any length of time. The talcum powder with which the inner and outer surface of rubber tubing is coated is probably the source of the trouble in the case of new rubber tubing. It is probably contaminated with ammonia.

Method.—To the decomposed blood filtrate in a large test tube (or cylinder) add a little paraffin oil and 1 or 2 c.c. of 10 per cent sodium hydroxide. The test tube is fitted with a two-hole rubber stopper and glass connections so arranged that air (previously free from ammonia, see page 44) is passed to the bottom of the cylinder and then through a special absorption tube in the receiving test tube, marked at 25 c.c., containing a little water and 2 c.c. of 0.1 N hydrochloric acid. Pass the air current through rather slowly for 1 minute, and then nearly as fast as the apparatus can stand for 10 to 15 minutes. Rinse the connecting tube; dilute the contents of the receiver to 20 c.c., add 2.5 c.c. of Nessler solution, dilute to the 25 c.c. mark, and make the color comparison in the usual manner.

Estimation of Preformed Creatinine

Method.[1, 6]—Transfer 25 (or 50) c.c. of a saturated solution of purified picric acid to a small, clean flask, add 5 (or 10) c.c. of 10 per cent sodium hydroxide and mix. Transfer 10 c.c. of blood filtrate to a small flask or to a test tube. Transfer 5 c.c. of the standard creatinine solution* to another flask, and dilute the standard to 20 c.c. Then add 5 c.c. of the freshly prepared alkaline picrate solution to the blood filtrate, and 10 c.c. to the diluted creatinine solution. Let stand for 8 to 10 minutes and make the color comparison in the usual manner never omitting first to ascertain that the two fields of the colorimeter are equal when both cups contain the standard picrate solution. The color comparison should be completed within 15 minutes from the time the alkaline picrate was added. For this reason it is never advisable to work with more than three to five blood filtrates at a time.

When the amount of blood filtrate available for the creatinine estimation is too small to permit repetition, it is of course advantageous or necessary to start with more than one standard. If a high creatinine should be encountered unexpectedly without several standards ready, the determination can be saved by diluting the unknown with an appropriate amount of the alkaline picrate solution—using for such dilution a picrate solution first diluted with two volumes of water—so as to preserve equality between the standard and the unknown in relation to the concentration of picric acid and sodium hydroxide.

Calculations.—The reading of the standard in mm. (usually 20) multiplied by 1.5, 3,

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**Folin's Creatinine Standard.*—One standard creatinine solution, suitable both for creatinine and for creatine determinations in blood, can be made as follows: Transfer to a liter flask 6 c.c. of a standard creatinine solution containing 1 mg. creatinine per c.c. (See page 212). Add 1 c.c. of pure concentrated hydrochloric acid, dilute to the mark with water, and mix. Transfer to a bottle and add four or five drops of toluene or xylene. Five c.c. of this solution contain 0.03 mg. of creatinine, and this amount plus 15 c.c. of water represents the standard needed for the vast majority of human bloods, for it covers the range of 1 to 2 mg. per 100 c.c. In the case of unusual bloods representing retention of creatinine, take 10 c.c. of the standard plus 10 c.c. of water, which covers the range of 2 to 4 mg. of creatinine per 100 c.c. of blood; or 15 c.c. of the standard plus 5 c.c. of water by which 4 to 6 mg. can be estimated. By taking the full 20 c.c. volume from the standard solution at least 8 mg. can be estimated; but when working with such blood, it is well to consider whether it may not be more advantageous to substitute 5 c.c. of blood filtrate plus 5 c.c. of water for the usual 10 c.c. of filtrate.

4.5 or 6 (according to how much of the standard solution was taken), and divided by the reading of the unknown in mm., gives the amount of creatinine in mg. per 100 c.c. of blood. In connection with this calculation it is to be noted that the standard is made up to twice the volume of the unknown, so that each 5 c.c. of the standard creatinine solution, while containing 0.03 mg., corresponds to 0.015 mg. in the blood filtrate.

Estimation of Creatine Plus Creatinine

Method.—With the aid of a pipette transfer 5 c.c. of the blood filtrate to a test tube graduated at 25 c.c., such as are used for the urea estimation. Add 1 c.c. of normal hydrochloric acid. Cover the mouth of the test tube with tinfoil and heat in the autoclave to 130° C. for 20 minutes or, as for the urea hydrolysis, to 155° C. for 10 minutes. Cool. Add 5 c.c. of the alkaline picrate solution and let stand for 8 to 10 minutes, then dilute to 25 c.c. The standard solution required is 20 c.c. of creatinine solution in a 50 c.c. volumetric flask. Add 2 c.c. of normal acid and 10 c.c. of the alkaline picrate solution and after 10 minutes standing dilute to 50 c.c. The preparation of the standard must of course have been made first so that it is ready for use when the unknown is ready for comparison.

Calculation.—The height of the standard, usually 20 mm., divided by the reading of the unknown and multiplied by 6 gives the "total creatinine" in mg. per 100 c.c. of blood. This may be converted into terms of creatine, by subtracting the preformed creatinine from the total creatinine and multiplying by 1.16.

In the case of uremic bloods containing large amounts of creatinine 1, 2, or 3 c.c. of blood filtrate, plus water enough to make approximately 5 c.c., are substituted for 5 c.c. of the undiluted filtrate. The normal value for "total creatinine" (and for creatine) given by this method is about 6 mg. per 100 c.c. of blood.

Estimation of Uric Acid

In the method described below Folin[5] has adopted several of the features recently introduced by Benedict (see page 61). Folin does not believe, however, that Benedict's new reagent is the fundamental factor in making the color reaction so highly specific for uric acid. He believes that this is chiefly due to the use of sodium cyanide as the sole alkali and for this reason still employs the original Folin-Denis uric acid reagent.

Method.[5, 6]—Fill a Pyrex 1 liter beaker, two-thirds full with water and heat to boiling. Transfer 5 c.c. of the blood filtrate and 2 c.c. of water to one test tube graduated at the 25 c.c. mark, and transfer 5 c.c. of the standard uric acid solution* and 2 c.c. of water to another similar test tube. Add 2 or 3 drops of 20 per cent lithium sulfate solution to each. From a burette add 2 c.c. of the 15 per cent sodium cyanide solution.† By means of a Folin 5 c.c. blood pipette, or other graduated pipette, add 1 c.c. of the Folin-Denis

**Folin's Uric Acid Standard.*—Transfer 100 mg. of uric acid to a clean 100 c.c. beaker. Dissolve 50 mg. of lithium carbonate in 20 c.c. of water in a large test tube or small beaker and heat to 60 to 70°C., and pour this warm alkali on the uric acid and stir until the uric acid is completely dissolved. As soon as a clear solution is obtained, add 5 c.c. of 40 per cent formaldehyde and then 1 c.c. of 50 per cent acetic acid. Transfer the solution quantitatively to a 100 c.c. volumetric flask and dilute to volume, mix and transfer to a bottle. This solution contains 1 mg. of uric acid per cubic centimeter.

To prepare the dilute standard for blood analysis, transfer 1 c.c., containing 1 mg. of uric acid, to a 250 c.c. volumetric flask. Half fill the flask with water; then add 10 c.c of two-thirds normal sulfuric acid, and 1 c.c. (but no more) of 40 per cent formaldehyde. Dilute to the mark with water and mix. This solution should keep for at least a month. Use 5 c.c., containing 0.02 mg. of uric acid for each determination.

†This is a 15 per cent solution of sodium cyanide in 0.1 N sodium hydroxide. The sodium hydroxide is used simply to increase the keeping quality of the cyanide.

uric acid reagent* to each test tube. Mix, and let stand for two minutes. At the end of 2 minutes transfer both test tubes to the boiling water bath and leave them there for 80 seconds. Cool, dilute to volume, and make the color comparison in the usual manner, not omitting first to read the standard against itself.

It will be noted that the heating period is given in terms of seconds. The reason for this limitation is the greater tendency which is further increased by the evaporation within the tube, whereby a faint ring of precipitate is necessarily formed at the surface of the liquid. Heating for 60 seconds in boiling water is sufficient to give the maximum color. There is, therefore, no object in heating longer than the time prescribed.

Calculation.—When the standard is set at 20 mm., 20 divided by the reading of the unknown, times 4, gives the uric acid content in milligrams per 100 c.c. of blood. The proportionality of color is in this case so good, that if the cyanide is right, readings between 10 and 40 mm., covering a range of 2 to 8 mg., are dependable.

If the uric acid content is too high (reading less than 10 mm.) repeat the determination with 3 c.c. of blood filtrate (plus 2 c.c. of water). If the blood filtrate is too weak, repeat the determination with 10 c.c. of filtrate and 5 c.c. of the standard (plus 5 c.c. of water). Such changes would obviously necessitate corresponding alterations in the calculation.

Folin states that the simple process described above gives substantially correct values for the uric acid content of the blood. Theoretically it should give too high values because the uric acid has not been separated from certain other (mostly unknown) products in the blood which give the same color reaction as uric acid. Under the conditions given (heat, weak alkalinity, and sodium cyanide as the only alkali) the color given by uric acid is, however, increased very much (10 to 12 times) and the color formerly obtained from other products in the blood is practically eliminated. Folin gives a modification of the Folin-Wu procedure, in which the uric acid is first separated from the other constituents as a check process.

Modified Folin-Wu Method.—Transfer from 2 to 5 c.c. (usually 5 c.c.) of the blood filtrate to a 15 c.c. centrifuge tube. With a blood pipette, or a small cylinder, add 7 c.c. of the silver lactate solution.† Allow to settle 1 to 2 minutes and then centrifuge. All the uric acid, down to the last trace, will now be in the precipitate (together with the chlorides). Decant the supernatant solution as completely as possible, and to the precipitate add 1 c.c. of a 10 per cent solution of sodium chloride†† in tenth normal hydrochloric acid. Stir thoroughly with a fine glass rod; add 4 c.c. of water and stir again. Centrifuge. Decant the last supernatant solution as completely as possible, but without rinsing, into a test tube graduated at the 25 c.c. mark. Transfer 5 c.c. of the standard uric acid solution (footnote, page 152) to another similar test tube, and then proceed with the determination exactly as in the first process described (page 152) not forgetting to have the boiling water ready at the right time.

**Folin-Denis Uric Acid Reagent.*—Transfer 100 gm. of sodium tungstate to a 2 liter flask and add 750 c.c. of distilled water. Shake until solution is obtained. A little white, fine, insoluble residue remains, due to presence of calcium. To the solution add 80 c.c. of 85 per cent phosphoric acid, ordinary phosphoric acid syrup. Close the mouth of the flask with a funnel plus two watch glasses, one small and one large, and boil gently but continuously for 2 hours. The color of the solution depends upon how much organic material is present. If it is very dark it may be bleached by the addition of a few drops of bromine, but this is usually superfluous. Boil for 10 to 15 minutes more to remove the surplus bromine. Cool and dilute to 1 liter.

†*Silver Lactate Sodium.*—Dissolve 100 gm. of silver lactate in about 700 c.c. of warm water. To 100 c.c. of 85 per cent lactic acid add 100 c.c. of 10 per cent sodium hydroxide. Pour this partly neutralized lactic acid into the silver lactate solution, dilute to 1 liter and set aside to allow the sediment, always present, to settle. Use only the clear supernatant solution. By thus neutralizing a part of the lactic acid, one provides for any excess acidity present in the blood filtrate and also for traces of mineral acids which may be present is some samples of silver lactate or in the lactic acid.

††*Sodium Chloride Solution.*—This solution contains 10 per cent of sodium chloride in 0.36 per cent hydrochloric acid. It may easily be prepared by adding 1 c.c. of pure concentrated hydrochloric acid (which should be 36-37 per cent) to 100 c.c. of 10 per cent sodium chloride solution.

Estimation of Amino Acid Nitrogen

Since the estimation of amino acid nitrogen has thus far furnished information chiefly of scientific value and further, since portions of the method are rather detailed, only the essential facts of the method will be given. For further details Folin's original paper[4] or his Manual[6] should be consulted.

The method depends primarily upon the use of β-naphthoquinone-sodium monosulfonate as a color forming reagent for amino acids. This reagent was employed by Herter some years ago in the estimation of indole, and Folin has found it very serviceable for amino acids. This quinone compound does not react with any of the ordinary nitrogenous waste products, except ammonia, and the amount of ammonia present in blood is so small as to be of practically no consequence in connection with the determination of amino acid nitrogen.

Method.[4, 6]—5 c.c. of the filtrate are adequate for the amino acid determination, but if the filtrate is abundant, 10 c.c. make the process perhaps a little more convenient (see below).

Transfer to a test tube (capacity 30 to 35 c.c.) 1 c.c. of the standard acid glycine solution* representing 0.07 mg. of nitrogen and add 3 c.c. of water. To another similar test tube add 5 c.c. of the blood filtrate. Add 1 drop of 0.25 per cent phenolphthalein solution to each. Add 1 c.c. of the 1 per cent sodium carbonate solution† to the standard and then add carefully, drop by drop, enough of the sodium carbonate solution to the blood filtrate until it has approximately the same pink color as the standard (3 or 4 small drops are usually required). Add another 5 c.c. of water to the standard; the volume of the standard is to be twice that of the blood filtrate. Then prepare a fresh 0.5 per cent solution of the sodium salt of β-naphthoquinone-sulfonic acid †† and add 2 c.c. of this solution to the standard and 1 c.c. to the blood filtrate. Shake a little to make the solutions uniform and set them aside in a completely dark cupboard and leave them there until the following day; that is, for 19 to 30 hours.

At the end of the time specified add an acetic acid-acetate solution§—2 c.c. to the

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**Standard Amino Acid Solution.*—The standard solution of amino acid used in blood analysis should contain 0.07 mg. of nitrogen per c.c. It is well to make up a stock solution containing 0.1 mg. per c.c. The solution is made with 0.1 N hydrochloric acid, and 0.2 per cent of sodium benzoate, in which amino acids seem to keep indefinitely. Glycine (glycocoll) is the amino acid which can probably be used to best advantage. Since glycine contains 18.7 per cent nitrogen, 268 mg. would be required to make 500 c.c. of a solution containing 0.1 mg. N per c.c. From the stock solution containing 0.1 mg. of nitrogen per c.c. the blood standard is made by diluting 70 c.c. with 0.1 N hydrochloric acid to a volume of 100 c.c. It is obvious that the amino acid employed as a standard should be of known purity.

†*Special Sodium Carbonate Solution.*—The required carbonate solution is made as follows: 50 c.c. of approximately saturated solution are diluted to a volume of 500 c.c. The strength of the resulting solution is determined by titrating 20 c.c. of 0.1 N hydrochloric acid with the carbonate, and with methyl red as indicator. On the basis of the titration value thus obtained, the carbonate solution is diluted so that 8.5 c.c. are equivalent to 20 c.c. of 0.1 N acid. The carbonate solution is about 1 per cent.

The correct degree of alkalinity is obtained when 1 c.c. of this sodium carbonate solution is added to 1 c.c. of amino acid solution, which at the same time is a 0.1 N solution of hydrochloric acid. The alkalinity is, therefore, represented by a mixture of carbonate and bicarbonate. A drop of phenolphthalein should always be used when working with amino acids of unknown and variable acidity. It is advisable that the alkalinity in the different solutions, the standard and unknowns, should be approximately the same, but there is no need of trying to make them exactly equal.

††*Fresh 0.5 Per Cent Solution of the Sodium Salt of β-Naphthoquinone-Sulfonic Acid.*—Owing to the difficulties in securing this quinone, Folin has described a reasonably simple synthesis, by which enough of this compound for several thousand amino acid determinations can be made in the course of two mornings. In solution β-naphthoquinone-sulfonic acid is gradually decomposed and the solution becomes visibly darker in the course of a few hours, particularly if not kept in the dark. For this reason only freshly prepared solutions should be used. The samples need not be weighed any more accurately than can be done on a small torsion balance, because a variation either way of 5 per cent makes no difference.

Transfer 100 mg. of the quinone to a small flask, add 20 c.c. of water and shake. Complete solution is obtained almost at once. For miscellaneous amino-acid determinations when 0.1 mg. of nitrogen is the standard, 3 c.c. of the reagent are taken; for 5 c.c. of blood filtrate only 1 c.c.

§*Special Acetic Acid-Acetate Solution.*—Dilute 100 c.c. of 50 per cent acid with an equal volume of 5 per cent sodium acetate solution. The presence of the sodium acetate in this solution serves two purposes: (1) to increase unmistakably the color of the quinone-amino acid derivative, and (2) to retard very much the onset of turbidity due to the liberation of sulfur from the added sodium thiosulfate. Both of these results are due to the weakened acidity of the acetic acid.

standard and 1 c.c. to the blood filtrate. After the acetic acid has been added (never before) add the thiosulfate solution*—2 c.c. to the standard and 1 c.c. to the blood filtrate. Finally add with a Folin blood pipette 14 c.c. of water to the standard giving a volume of 30 c.c. and add 7 c.c. of water to the blood filtrate (final volume 15 c.c.). Mix and make the color comparison, setting the standard at 20 mm.

Calculation.—20 divided by the colorimetric reading in mm., times 7; or 140 divided by the colorimetric reading, gives the amino acid nitrogen in milligrams per 100 c.c. of blood (it should be borne in mind that 5 c.c. of the blood filtrate corresponds to 0.5 c.c. of blood).

If 10 c.c. of blood filtrate can be spared for the amino acid determination, it is more convenient to make use of test tubes graduated only at 25 c.c.

The second process is more satisfactory than the first where the amino acid nitrogen content is unusually low, because, when the unknown is much weaker than the standard, the match in color is not very good, the weak one being very yellow.

With the first method when the amino acid content is low, it may be necessary to use a second standard containing 0.05 mg. of nitrogen.

Method.—Pipette 1 c.c. of the standard amino acid solution containing 1 c.c. of 0.1 N acid and 0.07 mg. of nitrogen into a test tube graduated at 25 c.c. and add 8 c.c. of water. To another such test tube add 10 c.c. of blood filtrate. Add 1 drop of phenolphthalein to each. Add 1 c.c. of the special 1 per cent sodium carbonate to the standard, and to the filtrate add the carbonate solution, drop by drop, until the color obtained matches approximately that of the standard. Add 2 c.c. of a freshly prepared 0.5 per cent β-naphthoquinone solution to each, mix, set aside overnight in a perfectly dark place. The next day add first 2 c.c. of the acetic acid-acetate solution and then 2 c.c. of the 4 per cent thiosulfate solution to each test tube. Dilute to the 25 c.c. mark, mix, and make the color comparison.

Calculation.—140 divided by the colorimetric reading, gives as above, the amino acid nitrogen in milligrams per 100 c.c. of blood.

Estimation of Blood Sugar

Method.[2, 6]—Pipette 2 c.c. of the tungstic acid blood filtrate into a Folin sugar tube, and to two other similar tubes (graduated at 25 c.c.) add 2 c.c. of standard sugar solution† containing respectively 0.2 and 0.4 mg. of glucose. To each tube add 2 c.c. of the alkaline copper solution.†† The surface of the mixtures must reach the constricted part of the tube. If the bulb of the tube is too large for the volume (4 c.c.) a little, but not more than 0.5 c.c., of a diluted (1:1) alkaline copper solution may be added. If this does not

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**Solution of Sodium Thiosulfate* ($Na_2S_2O_3.5H_2O$).—A 4 per cent solution of sodium thiosulfate is used to destroy the surplus quinone remaining after the full color obtainable from the amino acids has developed. It destroys the surplus color of the quinone and under the conditions prescribed has no effect on the colored quinone-amino acid derivative, at least during the first 1 or 2 hours. Nor do the colored solutions become turbid from liberated sulfur within the first 2 hour period.

†*Standard Sugar Solution.*—Dissolve 2.5 gm. of benzoic acid in 1 liter of boiling water and cool. Transfer to a bottle; the solution will keep indefinitely.

Dissolve 1 gm. of pure glucose in about 50 c.c. of the benzoic acid solution. Transfer to a 100 c.c. volumetric flask, rinse, and fill to the mark with the benzoic acid solution. This is the 1 per cent standard stock solution and appears to keep indefinitely.

Transfer 1 c.c. of the stock solution, by means of an Ostwald-Folin pipette to a 100 c.c. volumetric flask; fill to the mark with saturated benzoic acid and mix. The diluted solution so obtained, which contains 0.1 mg. of glucose per c.c., is a suitable standard for most blood sugar determinations. Another standard, twice as strong is occasionally needed. This is made by diluting 2 c.c. of the stock solutions to 100 c.c. with the benzoic acid solution. Use 2 c.c. for each determination.

††*Alkaline Copper Solution.*—Dissolve 40 gm. of anhydrous sodium carbonate in about 400 c.c. of water, and transfer to a liter flask. Add 7.5 gm. of tartaric acid, and when the latter has dissolved, add 4.5 gm. of crystallized copper sulfate; mix and make up to a volume of 1 liter. If the carbonate is impure a sediment may form after a time. If this happens decant the clear solution into another bottle.

suffice to bring the contents to the narrow part, the tube should be discarded. Transfer the tubes to a boiling water bath and heat for 6 minutes. Then transfer them to a cold water bath and let cool, without shaking, for 2 to 3 minutes. Add to each test tube 2 c.c. of the molybdate phosphate solution.* The cuprous oxide dissolves rather slowly, if the amount is large, but the whole up to the amount given by 0.8 mg. of glucose, dissolves usually in 2 minutes. When the cuprous oxide is dissolved, dilute the resulting blue solutions to the 25 c.c. mark, insert a rubber stopper, and mix. It is essential that adequate attention be given to this mixing because the greater part of the blue color is formed in the bulb of the tube.

The two standards, representing 0.2 and 0.4 mg. of glucose are adequate for practically all cases. They cover the range from about 70 to nearly 400 mg. of glucose per 100 c.c. of blood.

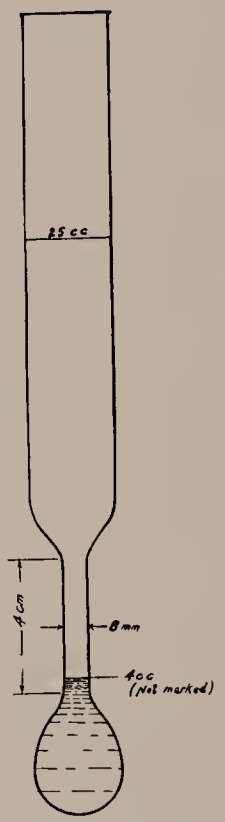

Fig. 18.—Folin's blood sugar tube.

Calculation.—The depth of the standard in millimeters (20) multiplied by 100, or by 200 if the stronger standard is taken, and divided by the colorimetric reading of the unknown gives the sugar content in mg. per 100 c.c. of blood.

Cooling of the alkaline cuprous oxide suspension before adding the phosphate molybdate solution is not essential, and in case of one or two determinations only, may be omitted. In a large series of determinations it is probably best to use it. The important point is that the standard and the unknowns should not only be heated the same length of time but should also have substantially the same temperature when the acid reagent is added. The maximum color develops faster in hot solutions; but if a reasonable

**Special Phosphomolybdic Acid Solution.*—Transfer to a liter beaker 35 gm. of molybdic acid and 5 gm. of sodium tungstate. Add 200 c.c. of 10 per cent sodium hydroxide and 200 c.c. of water. Boil vigorously for 20 to 40 minutes so as to remove nearly the whole of the ammonia present in the molybdic acid. Cool, dilute to about 350 c.c., and add 125 c.c. of concentrated (85 per cent) phosphoric acid. Dilute to 500 c.c.

uniformity of condition is maintained, it makes no difference whether the color comparison is made at the end of 5 minutes or at the end of one hour.

With the improved technic Folin and Wu state that reoxidations of the cuprous compounds are excluded, the blank due to the blue alkaline copper tartrate is eliminated, and, finally, the error due to the so-called phenols in blood filtrates is removed.

Micro-Estimation of Blood Sugar

Foster[9] has suggested a micro-modification of the Folin-Wu procedure, which makes feasible the estimation of blood sugar on finger blood. It should be remembered, however, that in this case one is really dealing with arterial blood (see page 89). According to Foster the micro adaptation is nearly as accurate as the original. For both methods he thinks it desirable to have a third standard of 0.3 mg. of glucose per 2 c.c. in addition to the 0.2 and 0.4 mg. standards employed by Folin and Wu.

Taking of Blood.—The hand if cold is immersed for a minute in warm water. The tip of a finger is then lanced with a spring lancet and the blood (0.3 c.c.) is collected directly into a pipette. The lancet is ground to a chisel-like cutting edge 1 mm. wide and is set to deliver a prick about 3 mm. deep. No stasis is used but sometimes the finger is gently "milked" if the flow is not sufficiently rapid.

Method.—The estimation is carried out exactly as described for the macro method, except that only 0.3 c.c. of blood is required.

The 0.3 c.c. of blood is pipetted into a short rather broad test tube and diluted with 2.1 c.c. of water and mixed to lake the blood. 0.3 c.c. of 10 per cent tungstic acid is then added and mixed with the blood, followed by 0.3 c.c. of ⅔ normal sulfuric acid (see page 145) shaking after each drop. The contents of the tube are now poured on a small filter paper just large enough to hold all at one time.

The sugar estimation is made on 1 c.c. of filtrate using a sugar tube just half the size of the one illustrated above.

Estimation of Chlorides

Folin[6] comments regarding the method of Whitehorn's,[3] included in his system, that this simple method is doubtless open to theoretical errors, but practically it seems to give accurate and dependable results.

Method.[3]—Pipette 10 c.c. of the protein-free (tungstic acid) filtrate (equivalent of 1 c.c. of blood) into a porcelain dish. Add with a pipette 5 c.c. of the standard silver nitrate solution* and stir thoroughly. Add about 5 c.c. of concentrated nitric acid (sp. gr. 1.42), mix and let stand for 5 minutes, to permit the flocking out of the silver chloride. Then add with a spatula an abundant amount of powdered ferric ammonium sulfate (about 0.3 gm.) and titrate the excess of silver nitrate with the standard sulfocyanate solution† until the definite salmon-red (not yellow) color of the ferric sulfocyanate persists in spite of stirring for at least 15 seconds.

**Standard Silver Nitrate Solution (M/35.46).*—Dissolve 4.791 gm. of C. P. silver nitrate in distilled water. Transfer this solution to a liter volumetric flask and make up to the mark with distilled water. Mix thoroughly and preserve in a brown bottle. 1 c.c. = 1 mg. Cl. (It is to be noted that the silver nitrate and nitric acid are not added to the protein free filtrate simultaneously. To do so may result in the mechanical enclosure of silver nitrate solution within the curds, and a consequent error in the positive direction.)

†*Standard Sulfocyanate Solution (M/35.46).*—Because sulfocyanates are hygroscopic, the standard solution must be prepared volumetrically. As an approximation about 3 gm. of potassium sulfocyanate or 2.5 gm. of ammonium sulfocyanate are dissolved in a liter of water. After ascertaining the strength of the sulfocyanate solution by titrating under the conditions described in the method, one may dilute the sulfocyanate so that 5 c.c. are equivalent to 5 c.c. of the silver nitrate solution.

Calculation.—Since each c.c. of the thiocyanate solution used is equivalent to 1 c.c. of silver nitrate solution, the difference between the volume of silver nitrate solution taken and the excess determined by the titration, is 5 – titer, represents the volume which reacted with chloride at the ratio of 1 c.c. to 1 mg. of Cl. The 10 c.c. of blood filtrate taken is the equivalent of 1 c.c. of blood (or plasma).

5.00–titer (in c.c.) = mg. of Cl per c.c. of blood (or plasma). To convert Cl figures into NaCl figures divide by 0.606.

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[6]Folin, O.: Laboratory Manual of Biological Chemistry, D. Appleton and Company, New York, 1922, pp. 229-291.

[7]Hiller, A., and Van Slyke, D. D.: A Study of Certain Protein Precipitants. Jour. Biol. Chem., 1922, liii, 253-267.

[8]Berglund, H.: Nitrogen Retention in Chronic Interstitial Nephritis and its Significance. Jour. Am. Med. Assn., 1922, lxxix, 1375-1380.

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CHAPTER X

MISCELLANEOUS BLOOD DETERMINATIONS

THERE are many chemical determinations on the blood, which, for the sake of brevity, have been omitted from the foregoing chapters of this little book. It is not believed that these methods furnish information of as great practical usefulness as those already described, still one may occasionally find it necessary to carry out some of these determinations. Methods will be given for the estimation of the total solids, total nitrogen, hemoglobin, oxygen capacity and content, acetone bodies, calcium and inorganic phosphorus, and references to papers describing the technic of various other determinations. The clinical significance of these different determinations is discussed.

Blood Volume.—Owing principally to the recent work of Keith, Rowntree and Geraghty[1] the subject of blood volume has received considerable attention. These investigators have introduced a new method of determining blood volume and have obtained somewhat higher figures than those formerly given for man. The principle underlying their method is the introduction directly into the circulation of a non-toxic, slowly absorbable dye (vital red) which remains in the plasma long enough for thorough mixing, and the determination of its concentration in the plasma colorimetrically by comparison with a suitable standard mixture of dye and serum. According to this method the plasma normally constitutes approximately 5 per cent, or one-twentieth of the body weight. The volume occupied by the corpuscles was calculated with the aid of the hematocrite and found to average 43 per cent for the erythrocytes and 57 per cent for the plasma. On this basis Keith, Rowntree and Geraghty have calculated that blood normally constitutes 8.8 per cent or 1/11.4 of the body weight. With this method they were able to demonstrate the amount of decrease in blood volume as the result of hemorrhage and of the increase following intravenous infusion of saline.

Significant observations were made in a few pathological conditions. Both the blood and plasma volume are increased in pregnancy, before term, but return to normal within a week or two after delivery. In obesity the plasma and blood volumes are relatively small. Many cases of anemia exhibit a relatively high blood volume while in some cases polycythemia in the sense of a high blood count may be dependent on a low plasma volume. In anasarca accompanying myocardial insufficiency the blood volume may be absolutely increased. In

TABLE I

DATA ON BLOOD VOLUME

From Bock, partly recalculated

CONDITION	NUMBER OF CASES	TOTAL PLASMA PER CENT OF BODY WEIGHT	TOTAL BLOOD PER CENT OF BODY WEIGHT	HEMOGLOBIN CALCULATED FROM O_2 CAPACITY PER CENT	RED BLOOD CELLS IN MILLIONS
Normal	5	5.1	8.2	16.4	4.8
Polycythemia	3	5.1	13.7	22.5	9.1
Pernicious Anemia	7	4.9	5.7	5.8	1.6
Miscellaneous	7	4.9	7.1	10.9	3.9
Diabetes	8	4.8	7.3	16.3	4.6

many cases of marked hypertension the volume is small, indicating that hypertension is not necessarily dependent upon a large blood volume.

The recent publication of Bock[2] on blood volume presents some very interesting data obtained with the vital red method on five normal and twenty pathological cases. The figures are given in Table I. The constancy of the plasma volume is pointed to as a striking fact, an observation not in entire harmony with the original observations of Keith, Rowntree and Geraghty. It will be noted in the table that although the plasma volume remains practically normal in polycythemia and anemia, the total blood volume is increased in the former and decreased in the latter owing to variations in the cell content.

Much work has recently been carried out on blood volume and the various methods have been criticized, but it seems to be generally conceded that the vital red method is as accurate as any and the best method at our disposal for most purposes.

Total Solids

Where a careful quantitative examination of the blood is being carried out, the estimation of the total solids is often of considerable value. In the

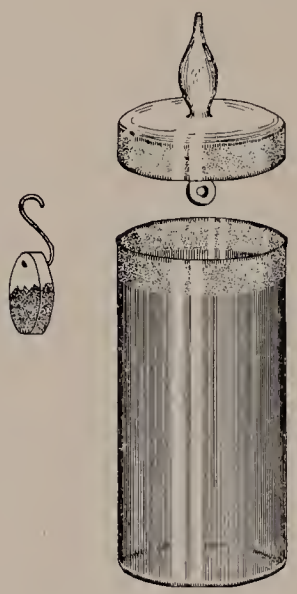

Fig. 19.—Author's weighing bottle for total solids in blood.

first place, the solid content of the blood is a very excellent index of the functional condition of the blood, blood proteins and blood cells taken together, and furthermore is of value in explaining small fluctuations in the content of the individual constituents. Normally the total solids amount to from 19 to 23 per cent, although in primary and secondary anemia, severe nephritis, etc., the amount may be decreased to nearly one-half these figures. That the total solids may be increased in cholera, as a result of the severe diarrhea, was recognized by Carl Schmidt many years ago. An increase in the blood solids was also found by Underhill to result from poisoning by the lethal war gases.

Method.—The total solids may be very simply and accurately determined[3] with the aid of the weighing bottle illustrated in Fig. 19. It is the ordinary style of bottle with stopper having a glass loop to which a block of filter paper may be fastened with a small wire hook. The block of filter paper is suspended and the bottle and the paper dried and weighed. From a small pipette 0.4 to 0.8 gm. of the well mixed blood is allowed to flow

on the filter paper rapidly, the stopper quickly inserted to prevent loss of moisture, and the bottle weighed. The stopper is now tilted and the bottle placed in the drying oven at 105° C. over night. The bottle is then cooled and again weighed. From the loss in moisture the total solids may readily be calculated. When very accurate figures for total solids are desired the blood should be taken without admixture of oxalate; but with the ordinary specimen in which 2 or 3 drops of 20 per cent potassium oxalate are present in 10 to 25 c.c. of blood, the error is about 1 to 3 per cent, i. e., the total solid figures are raised from 0.2 to 0.5 per cent.

Total Nitrogen

The total nitrogen content of perfectly normal blood amounts to somewhat more than 3 per cent. Of this, 99 per cent is derived from the various proteins of the blood, about three-quarters being from the cellular constituents, chiefly hemoglobin, and one-quarter from the plasma proteins, albumin, globulin and fibrinogen. The hemoglobin is obviously the most important as well as the most variable contributor to the total nitrogen. In pernicious anemia the total nitrogen may be reduced to considerably less than half the normal figure, while in severe nephritis the nitrogen content is frequently very low.

Method.[3]—One c.c. of the well-mixed oxalated blood is pipetted into a 25 c.c. volumetric flask with an Ostwald-Folin pipette. The blood is laked with distilled water and made up to volume. After thorough mixing, 1 c.c. of the diluted blood is pipetted with the Ostwald-Folin pipette into a thin glass test tube about 150 mm. in length and of diameter (20 mm.) such that it will readily slip into the 100 c.c. cylinder of the aeration apparatus. About 0.5 gm. of potassium sulfate, 2 drops of copper sulfate and 1 c.c. of concentrated sulfuric acid

Table II

Blood Serum Proteins in Health and Disease
Averages compiled from data of Rowe[4]

CONDITION	NUMBER OF CASES	ALBUMIN	GLOBULIN	TOTAL PROTEIN	GLOBULIN TO TOTAL PROTEIN
		per cent	per cent	per cent	per cent
Normal subjects	22	5.6	1.9	7.5	22.5
Syphilis	19	5.0	2.5	7.5	34
Pneumonia	8	3.7	2.5	6.2	40
Chronic nephritis with edema	3	2.5	1.7	4.2	40
Chronic nephritis with uremia	5	4.2	2.3	6.5	35
Chronic nephritis without uremia or edema	7	4.5	2.2	6.7	33
Cardiac decompensation	9	4.7	2.6	7.3	36
Arteriosclerosis	9	4.8	2.3	7.1	32
Diabetes	10	5.5	1.9	7.4	26
Anemia	9	3.9	1.7	5.6	30

added, and digestion, aeration and nesslerization carried out just as described in the case of nonprotein nitrogen (see page 43). Except in severe anemia, 0.04 c.c. of blood contains about the right amount of nitrogen for convenient nesslerization.

Plasma Proteins (Albumin, Globulin and Fibrinogen).—The subject of the serum proteins in man has recently been very carefully considered by Rowe,[4] who has employed the microrefractometric method of Robertson for their study in normal and a number of different pathological conditions. In a series of twenty-two normal cases the serum albumin was found to vary between 4.6 and 6.7 per cent, the serum globulin between 1.2 and 2.3

per cent, the total serum protein between 6.5 and 8.2 per cent and the nonproteins between 1.1 and 1.3 per cent, while the percentage of globulin in the total protein varied from 16 to 32 per cent. Muscular activity even of the simplest sort, increases total serum proteins, this increase occurring more in the albumin than the globulin fraction. In three cases with severe muscular work Rowe found the total protein increased from 1.1 to 1.9 per cent and the albumin from 0.8 to 1.5 per cent, while in one case with light exercise the total protein was increased 0.5 per cent and the albumin 0.3 per cent.

Table II on preceding page compiled from data given by Rowe gives a comparative idea of the blood serum proteins in the normal human subject and in a variety of pathological conditions.

From Table II it is apparent that in syphilis the globulin is definitely increased, while the total protein remains about normal. In pneumonia the globulin is increased more in relation to the total protein than in syphilis, while the total protein is reduced, due probably in large measure to a dilution of the blood serum by water retention, which occurs in fever. The lowest values for total serum proteins are obtained in chronic nephritis with edema, due probably to chronic intoxication as well as hydremia. In this type of cases (nephrosis) Epstein[5] has found that there is not only a reduction in the total protein, but also that the globulin is both absolutely and relatively increased, constituting as much as

TABLE III

BLOOD PROTEINS* AND CHOLESTEROL IN CASE OF NEPHROSIS

DATE 1923	PROTEINS				CHOLESTEROL	UREA N	CHLORIDES AS Na Cl
	TOTAL SERUM PROTEINS	ALBUMIN	GLOBULIN	FIBRIN			
	per cent	per cent	per cent	per cent	per cent	mg. to 100 c.c.	per cent
July 9					0.307	20	0.488
" 18					0.328	12	0.525
" 23					0.322	26	0.513
" 27	3.97	1.40	2.57	0.83		43	0.525
" 30	4.45	1.93	3.52	0.45	0.315	39	0.500
Aug. 6	4.62	1.98	2.64	0.70	0.390	18	
" 13	4.84	1.44	3.40	0.47	0.316	14	0.550
" 20	4.50	2.14	2.36	0.51			
" 27	5.36	2.69	2.67	0.61			
Sept. 4	5.27	3.70	3.70	0.62	0.500	41	0.550
" 10	5.73	3.42	3.42	0.79	0.448	31	

*Unpublished analyses of Killian made with the Wu method.

Case L.L., Chinese male, aged 36. Salt free diet July 17-28, high protein salt free diet July 28-Sept. 11.

90 per cent of the total protein. A case of nephrosis nicely illustrating this point is shown in Table III. In chronic nephritis with uremia the total proteins may be nearly normal but the globulin is usually increased. Except in very severe diabetes the findings are practically normal. In pernicious anemia the total proteins are not as low as would be expected from examination of the whole blood, being higher than in nephritis with edema.

According to Whipple[6] the normal fibrinogen limits for the human subject may be given as 0.3 to 0.6 per cent with an average of 0.5 per cent per 100 c.c. of plasma. In pneumonia and septicemia fibrinogen is much above normal, reaching 0.9 per cent while in acute liver injury it drops to a very low level or even zero in some fatal cases. In chronic liver disease fibrinogen often falls markedly and may cause bleeding (cirrhosis). In general cachexias, such as sarcomatosis, nephritis and miliary tuberculosis, the fibrinogen may be quite low, 0.1 per cent.

In addition to the microrefractometric method of Robertson[7] for the estimation of the serum proteins, referred to above, Cullen and Van Slyke[8] have quite recently described methods of determining the fibrin, globulin and albumin nitrogen of blood plasma. Their method has since been modified and simplified by Howe,[9] while very recently Wu[10] has proposed a simple colorimetric procedure of estimating the plasma proteins.

Hemoglobin

The estimation of hemoglobin was apparently the first chemical determination in the blood to find extensive clinical application. It seems unfortunate that most of the estimations recorded should have been made employing an empirical scale with 100 as the normal, especially since the 100 is somewhat of a variable factor with different methods owing to different standardizations. Of the older clinical instruments the Fleischl-Miescher is standardized to give the actual percentage of hemoglobin. Sahli states that in his original instrument the 100 was designed to correspond with an actual hemoglobin percentage of 17.2 per cent. Haldane[11] employs as a standard of 100 a 1 per cent solution of a blood having an oxygen capacity of 18.5 per cent. Such a blood contains approximately 14 grams of hemoglobin per 100 c.c., a quantity rather below that found in normal adult blood by most workers. Haldane states that with normal women he found an average oxygen capacity of 16.5 per cent and with children 16.1 per cent, thus necessitating a different standard of 100 for women and children. Employing the Van Slyke method for oxygen capacity, Haden[12] in 1922 obtained an average hemoglobin of 15.83 gm. per 100 c.c. on 20 males between 18 and 30 years of age, 15.23 gm. on 20 males between 30 and 50 and 13.34 gm. on 12 females between 20 and 40. Recalculating his figures on the basis of 5 million red cells he obtained an oxygen capacity of 20.85, equivalent to 15.57 gms. hemoglobin. He suggests that all hemoglobinometers should be calibrated with the Van Slyke apparatus on this basis, so that 100 per cent equals 15.6 gm. per 100 c.c. of blood. Newcomer[13] has recently taken 16.92 gms. of hemoglobin per 100 c.c. of whole blood, the average obtained by Williamson[14] for adult males in Chicago, as his standard of 100.

The hemoglobin content of blood varies widely not only in disease but also during different age periods, as pointed out by Williamson. For these reasons it is much more logical to record hemoglobin as we do other blood determinations in grams per 100 c.c. or actual per cent Williamson has taken great pains to definitely establish the normal hemoglobin content of human blood of both males and females for the different age periods. His figures for these different periods were made with the accurate spectrophotometric method and were the average values found on fifteen or more individuals. It was ascertained that during the first two weeks of life the hemoglobin content exceeds 20 per cent, but then drops rather abruptly about the third month to below 14 per cent and does not pass this figure until the tenth year. During the adult period of life in both sexes (from 16 to 70 years) the hemoglobin maintains a fairly constant level of slightly above 16 per cent. From the third month to the fifteenth year the values obtained in the female appear to slightly exceed the male, although from 16 to 60 the reverse is true, the hemoglobin of the female averaging close to 15.5 per cent, while in the male it reaches nearly 17 per cent. These age and sex variations are well brought out in Fig. 20 taken from Newcomer's compilation of Williamson's data.

As pointed out above Bock has recently made the very interesting observation that the blood plasma volume is very constant under normal and a great variety of pathological conditions, forming about 5 per cent of the body weight. Since the plasma volume is practically constant, the difference in the blood volume in these two conditions is obviously dependent upon differences in cell content, chiefly in that of the hemoglobin carrying red cells. His normal cases showed a hemoglobin content of 16.4 per cent, whereas in pernicious anemia it was 6.5 per cent and in polycythemia 22.5 per cent.

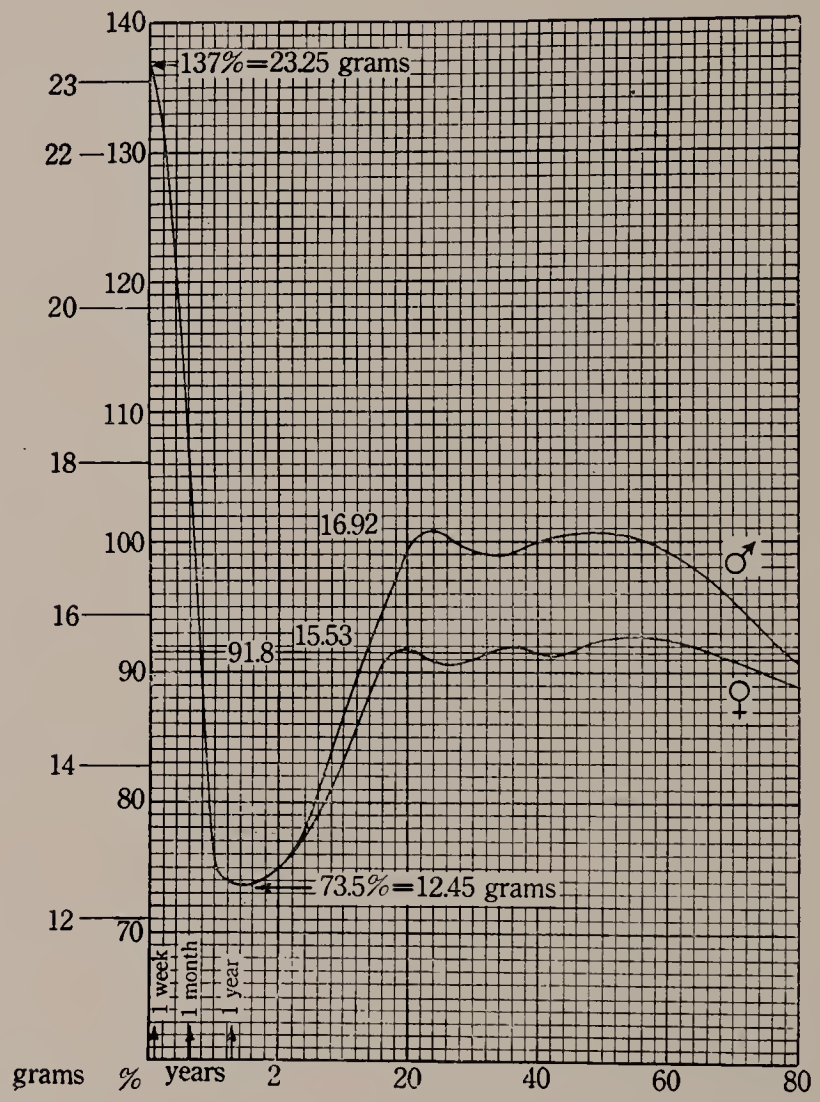

Fig. 20.—Newcomer's graphic presentation of Williamson's hemoglobin data for age periods.

Normally the total solids of the blood amount to about 21 per cent, and of this fully 16 per cent is accounted for by the hemoglobin. This being the case, hemoglobin is by far the most important variant in pathological bloods. Consequently its estimation should prove of considerable value as a part of routine chemical blood analyses.

Excepting such blood diseases as pernicious anemia and chlorosis, where the hemoglobin content of the cells may be increased or decreased respectively, thus giving rise to a high or low so-called "color index," the hemo-

globin parallels fairly closely the number of red cells, and consequently furnishes little added information. For this reason, if one excepts the conditions mentioned, a hemoglobin estimation might more logically supplement a chemical blood analysis than a blood count.

Owing to the increased number of colorimetric methods now in common use, especially in connection with the chemical analysis of blood, a standard colorimeter (see page 199) has become a part of the necessary equipment of every clinical laboratory at the present time. This being the case there is no reason why the same instrument should not be utilized for the estimation of hemoglobin. Adaptations of the Hoppe Seyler-Haldane carboxyhemoglobin method and the acid hematin method of Sahli have already been made to these instruments. Obviously the accuracy is far greater than with the microcolorimeters ordinarily employed for hemoglobin estimation.

In the past one of the greatest difficulties in the way of making an accurate colorimetric hemoglobin estimation has been to secure a correct standard. This is now in a fair way of solution owing to the development by Van Slyke[15] of a simple gasometric method of determining the oxygen capacity of the blood. Van Slyke employs the same gas burette as in his CO_2-combining power estimation, and this instrument is now in nearly every clinical laboratory. This method furnishes the basis of quickly obtaining a correct hemoglobin standard, since the oxygen capacity of blood is dependent upon its hemoglobin content (see method below). We have long known from the observations of Hüfner that one part of hemoglobin combines with 1.34 volumes of oxygen.

Palmer[16] has described a modification of the Haldane carboxyhemoglobin method which may readily be made on the oxalated bloods employed in chemical analyses. He states that with this method estimations may be made in two minutes time to an accuracy within about one per cent. The quantity of blood required (0.05 c.c.) is so small that satisfactory results may also be obtained when the usual clinical method of obtaining small amounts of blood by pricking the ear or finger tip is carried out with proper care. The chief objection to this method is that standards keep only a limited period.

When care is taken several workers have recently shown that satisfactory results may be obtained with the Sahli acid hematin method. Three serious criticisms may be made of this method as it is ordinarily carried out clinically: The standards are often inaccurate, the dilution colorimeter employed is too small to yield accurate results, and sufficient time is not generally allowed for the color to develop. Smith and Cohen[17] have shown, however, that with proper care quite as accurate results may be obtained with this as the Palmer method. Robscheit[18] has likewise pointed out that when at least one hour is allowed for the color to develop, she was able to obtain as consistent results as with the Palmer method. Berman[19] has also shown that heating for one minute will accomplish the same purpose. As a standard Newcomer[20] employs a colored glass plate, which may be inserted on the top of the plunger of the colorimeter. Although these glass standards are obviously permanent, they have the disadvantage that the plate requires careful standardization and

does not match perfectly the acid hematin in all concentrations. Very recently Newcomer[13] has embodied this principle in a special instrument constructed on the Duboscq pattern (see Fig. 21). This new instrument is very neatly constructed and should yield very accurate results. It should serve most satisfactorily for use in connection with cell counts, but for those doing chemical blood work and regularly employing a standard colorimeter, the instrument would appear to be an unnecessary duplication, since the colored glass plate, as Newcomer[20] originally pointed out, may be employed in connection with a standard colorimeter. It seems unfortunate that Newcomer has taken 100 as his normal standard (equivalent to Williamson's 16.92 grams per 100 c.c., the average of normal adult males), instead of graduating it to read grams per 100 c.c.

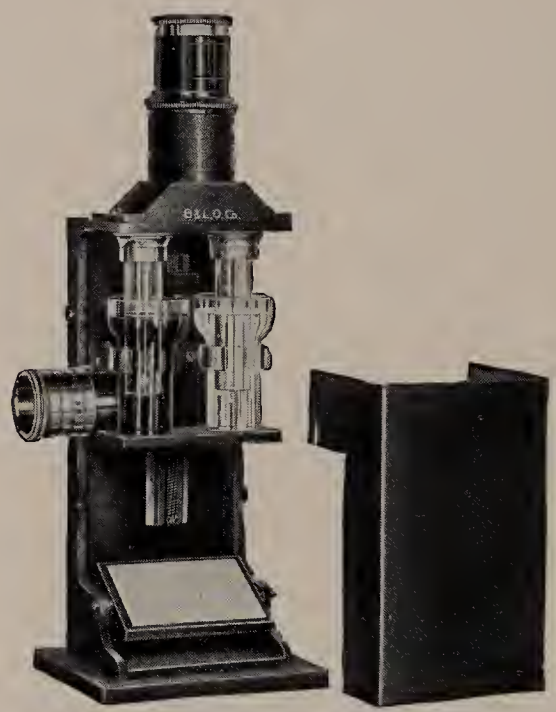

Fig. 21.—Newcomer's clinical hemoglobinometer.

The time honored hemoglobin test will yield results of greater clinical value when more attention is given to accurate standards and methods, and the results then computed to give the actual percentage of hemoglobin rather than the relation to an indefinite normal in parts per 100.

Until quite recently we have employed a comparatively strong standard of acid hematin for our hemoglobin estimations (an equivalent of 0.075 per cent of hemoglobin), necessitating a 1 to 200 dilution of the unknown since this furnishes a depth of color that can be very accurately matched. Owing to the fact, however, that even the concentrated acid hematin solutions (3.75 per cent) slowly deteriorate on standing, and consequently require frequent rechecking, we have finally adopted Newcomer's[20] suggestion of using a colored glass plate for our standard. These may be obtained from Bausch and Lomb. We have found that when one of these glass plates is inserted on the top of the left hand plunger of their biological

colorimeter, it makes a simple but most excellent hemoglobinometer. The insertion or removal of the glass disk requires only a few seconds. The filter is of special yellow glass chosen for its spectral properties. The glass disks are 1.0 mm. in thickness and Bausch and Lomb state that the thickness of the filter is controlled to 0.01 mm. making unnecessary any correction for filter thickness. Newcomer found that this thickness of filter was equivalent to 0.038 per cent of hemoglobin. We have very carefully checked the plate we are using with three separate solutions of acid hematin standardized by the Van Slyke oxygen capacity method, given below, and found it to be equivalent to an 0.0384 per cent solution of hemoglobin, a difference of only 1 per cent. It will be observed that this standard is half the strength of the standard we formerly employed, and consequently requires a blood dilution of 1 to 400.

Method.—By a gentle rotary motion the cells and plasma in the blood bottle are thoroughly mixed, after which 0.025 c.c. (0.05 c.c. in severe anemia) are pipetted into a test tube containing 10 c.c. of 1 per cent hydrochloric acid (10 c.c. of the concentrated acid to 1000 c.c. of water) and the pipette twice washed out with the acid. After mixing this tube is allowed to stand for two hours or longer, i. e., until the remainder of the chemical blood analysis is completed before reading. It has long been recognized that the full acid hematin color does not develop for some time. Newcomer[20] has given a time correction table, which shows that results are about 4 per cent low at the end of 10 min., 3 per cent at the end of 15 min., 2 per cent at the end of 20 min. and 1 per cent at the end of 40 min.

To match with the standard glass plate, insert this in the top of the left hand plunger of the colorimeter, pour a few c.c. of water in the cup and set at about the 10 mm. mark. Now partially fill the right hand cup with the unknown acid hematin solution and match against the standard.

Owing to the fact that the glass is perfectly clear, whereas the acid hematin solutions are slightly cloudy, the color match is not perfect. Nevertheless after one has had a little experience in checking against standard acid hematin solutions it is possible to obtain very reliable figures.

Calculation.—Although it is probably well to recalibrate the glass plates, they may be assumed to be the equivalent of a 0.038 per cent hemoglobin solution. This being the case, $\frac{10}{R} \times 0.038 \times$ dilution = grams of hemoglobin per 100 c.c. of blood.

For example, with a reading of 11.1 mm. and a dilution of 400 the formula would work out, $\frac{10}{11.1} \times 0.038 \times 400 = 13.7$ grams hemoglobin per 100 c.c.

Oxygen Content and Oxygen Capacity

As pointed out above, the ability of the blood to absorb and take up oxygen depends upon its hemoglobin content. Since hemoglobin so readily takes up and gives off oxygen, it is obvious that venous blood should be partly unsaturated and therefore differ from the arterial blood in respect to its oxygen content, and further that blood obtained from different parts of the venous system should differ in its oxygen unsaturation. In the human adult the superficial veins of the limbs and neck, particularly of the arms (vena medina), are the only sources from which venous blood can be obtained. This means that in the human only blood coming from a limited region, consisting chiefly of muscle, can be studied.

Lundsgaard[21] found that on twelve normal resting adults the oxygen

content of the venous blood ranged from 9.6 to 18.0 volumes per cent with an average of 13.6, while the oxygen unsaturation ranged from 2.7 to 9.0 volumes per cent with an average of 5.8. In studying this question on circulatory disorders he found that in twelve patients with compensated heart lesions the unsaturation fell within normal limits, between 2.5 and 8 volumes per cent, while in four patients with uncompensated heart disease the values for the unsaturation were all above the normal limits, from 9.7 to 15.2 volumes per cent. In such cases the oxygen unsaturation appears to afford an objective criterion of the positive effect of digitalis therapy. From studies performed on patients with varying amounts of hemoglobin it has been shown that the oxygen unsaturation of the venous blood is independent of the oxygen capacity, unless the latter is reduced below the normal value for oxygen unsaturation (about 5 volumes per cent). Lundsgaard found, for example, that in a polycythemic patient with an oxygen capacity of 33.4 volumes per cent, the venous oxygen unsaturation was 5.4 volumes per cent, while in an anemic patient with an oxygen capacity of 6.7 volumes per cent, the venous oxygen unsaturation was 5.2 volumes per cent, indicating that the tissues extract from the blood all the oxygen they need with apparently equal readiness, regardless of whether the extraction leaves a great oxygen reserve as in polycythemia, or practically no reserve as in anemia.

Considerable additional information may also be obtained when the study of the oxygen content of the arterial blood is included. Such studies have been conducted on normal and certain pathological conditions by Stadie and by Harrop, the arterial blood being obtained from the radial artery. Observations obtained by Stadie[22] for the arterial and venous oxygen, and total oxygen capacity of five normal resting men averaged as follows: oxygen capacity, per 100 c.c. of blood, 21.2 c.c., arterial oxygen content, 20.2 c.c., and venous oxygen content, 15.6 c.c. This gives an arterial unsaturation of 1.0 c.c. or 5.0 per cent and a venous unsaturation of 5.6 c.c. or 26.8 per cent. Similar studies were made on a series of pneumonia cases (chiefly post influenza), a high arterial unsaturation being observed in the fatal cases. A definite relation was found to exist between the degree of cyanosis and the per cent of arterial unsaturation. With increasing cyanosis the arterial unsaturation becomes greater. The venous saturation varies similarly. Obviously the cyanosis of pneumonia patients is due to the incomplete saturation of venous blood with oxygen in the lungs. The range of arterial and venous unsaturation encountered in fatal and nonfatal cases of pneumonia is well illustrated in Table IV, compiled from Stadie. As will be noted the

TABLE IV

THE OXYGEN UNSATURATION OF ARTERIAL AND VENOUS BLOOD IN PNEUMONIA

TYPE OF CASES	NO. OF CASES	ARTERIAL UNSATURATION			VENOUS UNSATURATION		
		MAX.	MIN.	MEAN	MAX.	MIN.	MEAN
Normal individuals	5	6.5	2.8	5.0	33.0	22.7	26.8
Nonfatal cases	16	33.0	1.6	13.9	61.2	14.4	36.3
Fatal cases	16	68.2	14.1	32.0	85.5	22.3	57.0

arterial unsaturation of the fatal cases averaged 32 per cent and in one case reached 68 per cent, the venous unsaturation exceeding 85 per cent.

The oxygen content of the arterial blood in anemia and heart disease has been studied by Harrop,[23] who likewise made a careful study of the blood gases (oxygen and carbon dioxide) in both the arterial and venous blood of fifteen normal subjects, his figures for oxygen agreeing closely with those of Stadie. With severe anemia the saturation of the arterial blood did not differ from the normal. Low absolute values were found for the oxygen content of the venous blood, but the normal oxygen consumption was maintained. No deviations from the normal were found in arterial and venous blood from cardiac patients without arrhythmias, well compensated, and at

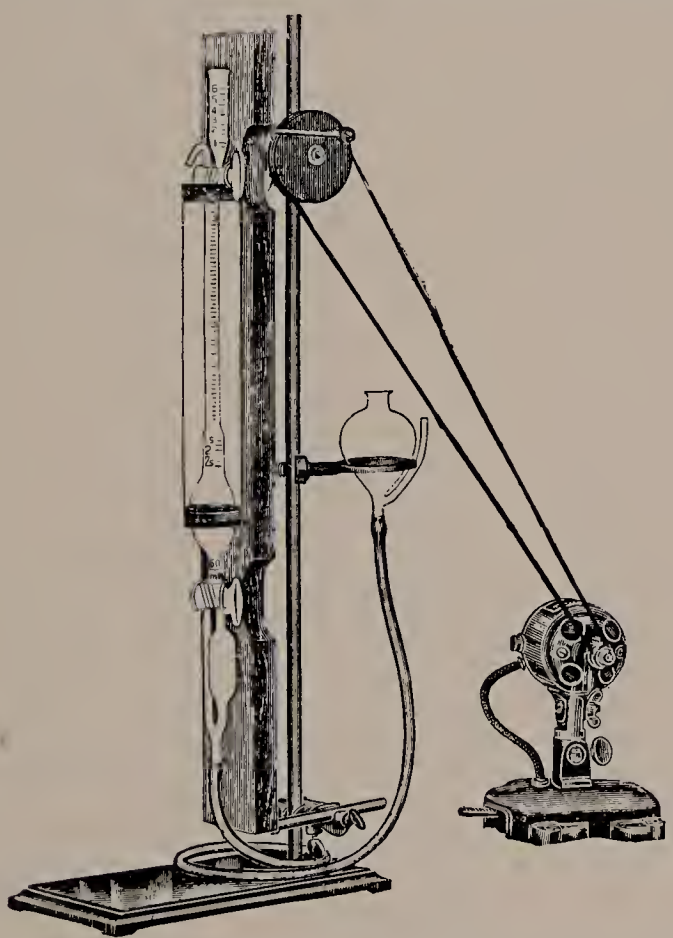

Fig. 22.—Van Slyke-Stadie water jacketed gas analysis apparatus with mechanical shaker.

rest in bed. With cardiac cases showing varying degrees of decompensation the arterial unsaturation is frequently abnormally low (sometimes exceeding 15 per cent), although not so low as that found in pneumonia. It is apparent that in many circulatory diseases during decompensation, particularly when there are physical signs of pulmonary congestion, there is a disturbance of the pulmonary exchange, as indicated by the lowering of the percentage saturation of the arterial blood with oxygen.

Since the oxygen capacity of blood depends upon its hemoglobin content, an important use of the oxygen capacity determination is in connection with the preparation of an accurate hemoglobin standard.

Method of Determining the Oxygen Capacity.—The Van Slyke[15] apparatus used for determining the CO_2-combining power of plasma may be used with equal facility for determining the oxygen-combining capacity (hemoglobin) of blood.

(Van Slyke has designed a new blood gas burette, which he has designated the constant volume apparatus. With this apparatus the gas is brought to 2 c.c. volume under diminished pressure, and its volume at atmospheric pressure calculated from the mm. Hg pressure. The accuracy of the determination with this new technic is materially increased. The method will be described by Van Slyke shortly.)

The apparatus is tested for tightness and freedom from gases as in the determination of CO_2 content or combining power. The entire apparatus is filled with mercury including the capillaries above the upper stopcock. For 2 c.c. of blood, 6 c.c. of water, 0.3 c.c. of 1 per cent saponin (Merck) solution and 2 or 3 drops of caprylic alcohol are introduced into the apparatus and freed of air, the extraction being repeated until no more air is obtained. The air-free solution is now drawn down and trapped in the wide branch of the apparatus below the lower stopcock (Lundsgaard). The stopcock is turned and mercury run very slowly upwards through the apparatus in order to collect the film of water left on the inside and this film expelled through the outlet capillary on the left side. Mercury is now run into the bottom of the cup and any moisture in the cup is dried by filter paper. Two c.c. of oxalated blood are now drawn into the 50 c.c. chamber from a pipette, by lowering the mercury reservoir, and trapped near the bottom of the chamber. While the upper stopcock remains open, the apparatus is shaken for ½ minute by hand or for from 2 to 3 minutes by a mechanical shaker (see Fig. 22) thus saturating the blood with oxygen. The mercury is run up again into the 50 c.c. chamber collecting the blood at the top. When the blood reaches the upper stopcock, this is closed. The lower stopcock is turned so that the previously trapped air-free water is allowed to rise in the chamber. The lower stopcock is closed and the apparatus (if not water jacketed and not fitted with shaker) turned upside down once in order to mix the water and blood. The blood is laked in ½ minute. From 0.10 to 0.12 c.c. of potassium ferricyanide solution (20 gms. per 100 c.c.) are measured into the cup and introduced into the chamber with the laked blood. A mercury seal is made and the apparatus is evacuated and shaken for 2 minutes. The evolved gas is composed of $O_2+N_2+CO_2$. The CO_2 is absorbed by introducing under slightly negative pressure 0.5 c.c. of 0.5 N NaOH and allowing it to trickle slowly down the inner wall of the chamber. The fluid mixture is trapped in the wide arm below the lower stopcock and mercury run up through the left arm and reading made in same manner as in the determination of CO_2 (see page 111).

The calculation of hemoglobin from oxygen capacity determined in this manner includes correction for temperature, barometric pressure and physically dissolved O_2+N_2. The oxygen combined with 100 c.c. blood under standard conditions multiplied by the factor 0.746 will give the grams of hemoglobin per 100 c.c. blood (see Table V).

Using a blood analyzed for hemoglobin (oxygen capacity) as a source of acid hematin standard, suitable solutions are made containing 3.75 per cent hemoglobin in 0.1 N HCl and keeping this as a stock solution and a second standard made by dilution with 0.1 N HCl to 0.075 per cent hemoglobin. The 0.075 per cent standard will be found suitable for determining the hemoglobin content of most bloods (see hemoglobin above). As pointed out above the colored glass plate of Newcomer, which we now use, is the equivalent of a 0.038 per cent solution of acid hematin in terms of hemoglobin.

Method of Determining the Oxygen Content.—With but slight differences the technic for the determination of oxygen content of blood is the same as for oxygen capacity. (Oxygen unsaturation is defined by Lundsgaard (1918) as the difference between the oxygen capacity and oxygen content of blood.)

The blood is collected out of contact with air; this may be accomplished by the use of mineral oil. For 2 c.c. of blood, 6 c.c. water, 0.3 c.c. 1 per cent Saponin (Merck) solu-

TABLE V

FACTORS FOR CALCULATION (VAN SLYKE AND STADIE[15])

TEMPERATURE	$f = \frac{B - w}{760\ (1 + 0.00367\ t)}$ factor by which gas measured moist at $t°$, B mm. is reduced to 0°, 760 mm.*	CO_2 a'	Air, † measured at room temperature and pressure, dissolved by		$1.017\ f\ (1 + \frac{S}{50 - S}\ CO_2\ a')$, factor by which the volume of CO_2 obtained after 1 extraction is multiplied in order to obtain the volume of CO_2, reduced to 0°, 760 mm., contained in the solution analyzed.	
			2.5 c.c. H_2O.	5 c.c. H_2O.	$S = 2.5$ c.c.	$S = 5.0$ c.c.
°C.			c.c.	c.c.		
15	$0.932 \times \frac{B}{760}$	1.075	0.052	0.105	$1.002 \times \frac{B}{760}$	$1.061 \times \frac{B}{760}$
16	0.928 "	1.043	0.051	0.101	0.995 "	1.053 "
17	0.924 "	1.015	0.050	0.100	0.989 "	1.046 "
18	0.919 "	0.989	0.049	0.098	0.983 "	1.038 "
19	0.915 "	0.966	0.048	0.096	0.978 "	1.030 "
20	0.910 "	0.942	0.047	0.095	0.972 "	1.022 "
21	0.906 "	0.919	0.046	0.093	0.966 "	1.015 "
22	0.901 "	0.896	0.045	0.091	0.960 "	1.008 "
23	0.897 "	0.873	0.045	0.090	0.954 "	1.001 "
24	0.892 "	0.850	0.044	0.088	0.948 "	0.993 "
25	0.888 "	0.828	0.043	0.086	0.942 "	0.986 "
26	0.883 "	0.808	0.042	0.084	0.936 "	0.978 "
27	0.878 "	0.789	0.041	0.083	0.931 "	0.971 "
28	0.873 "	0.772	0.040	0.081	0.924 "	0.964 "
29	0.868 "	0.755	0.040	0.080	0.918 "	0.957 "
30	0.863 "	0.738	0.039	0.078	0.912 "	0.950 "

*To calculate O_2 or hemoglobin when $O_2 + N_2$ volume is measured, multiply gas volume by f, to reduce to 0°, 760 mm., and by such factor as is necessary (100 when 1 c.c. of blood is used, 50 when 2 c.c. are used) to bring results to volume per cent basis. Then for

a. O_2 content, subtract ..... 1.36 vol. per cent N_2
b. O_2 bound by hemoglobin in venous blood, subtract..... 1.5 " " " " " " " "
c. O_2 bound by hemoglobin in arterial blood, subtract..... 1.7 " " " " " " " "
d. O_2 bound by hemoglobin in blood saturated with air at 20°, subtract ..... 2.1 " " " " " " " "

Grams of hemoglobin per 100 c.c. of blood $= 0.746\ d$.

Per cent of total hemoglobin saturated with $O_2 = \frac{100\ b}{d}$ or $\frac{100\ c}{d}$.

Volumes per cent O_2 unsaturation $= d - c$ or $d - b$.

b and *c* may be determined with slightly greater accuracy with the aid of Table V. The values for f given in the second column are for barometric readings corrected for temperature. The values for a'_{CO_2} are obtained by multiplying by $1 + 0.00367\ t$ the values for a_{CO_2} given by Bohr and Bock (1891).

†The dissolved air is given as measured at room temperature. It is subtracted from the air + CO_2 volume, measured after one extraction of plasma or aqueous carbonate solution, in order to obtain the CO_2 which is then multiplied by $1.017\ f \left(1 + \frac{S}{50 - S} a'_{CO_2}\right)$ in order to obtain the total volumes per cent of CO_2 in the solution analyzed. When whole blood is analyzed, the air correction cannot be used, because of the O_2 present, and the CO_2 must be determined by absorption with NaOH solution. The volume of gas absorbed is then multiplied by the above factor.

The factor 1.017, being empirical, may vary slightly for different apparatus.

tion and 2 or 3 drops of caprylic alcohol are introduced into the apparatus and freed of air. Nearly the entire 6 c.c. are then forced up into the cup of the apparatus. The blood is stirred to assure even distribution of corpuscles and drawn into a pipette calibrated to deliver 2 c.c. between two marks. (The author employs an Ostwald-Folin pipette with double graduation, see page 112.) The blood is allowed to flow from the pipette under the water solution in the cup, meanwhile the stopcock is partially opened so that the blood accompanied by some of the water flows at once into the chamber of the apparatus. The layer

of blood need never rise more than 1 or 2 mm. above the bottom of the cup, and the slight amount adhering is washed completely into the chamber by the water which follows after all the blood has been delivered.

Before the last c.c. of water is readmitted, 0.10 to 0.12 c.c. of a solution containing 20 gm. of potassium ferricyanide per 100 c.c. is added and thereby introduced into the chamber after the blood. A mercury seal is made and the remainder of the technic carried out in the same manner as that for oxygen capacity. The volume of gas measured is O_2+N_2 and correction must be made for temperature, barometric pressure and N_2 according to Table V, Factors for Calculation. From the volume of O_2+N_2 per 100 c.c. blood reduced to standard conditions a constant of 1.36 volumes is subtracted in calculating the oxygen content alone.

Methemoglobin.—Methods of methemoglobin estimation have recently been described by both Stadie[24] and by McEllroy.[25] Clinically it is occasionally helpful to be able to ascertain the methemoglobin content of blood, if any, especially after poisoning with certain drugs. In the Stadie method both hemoglobin and methemoglobin are changed quantitatively to cyanhemoglobin by a dilute solution of potassium cyanide. The color of the latter is a very brilliant orange-red and is very suitable for colorimetric comparison. The total amount of hemoglobin plus methemoglobin having been thus determined colorimetrically, the hemoglobin content of the blood containing the two pigments (hemoglobin and methemoglobin) is determined separately from the oxygen capacity, employing the Van Slyke method given above. The hemoglobin determined by the oxygen capacity is subtracted from the hemoglobin plus methemoglobin determined together as cyanhemoglobin; the difference is the methemoglobin.

Carbon Monoxide.—Van Slyke and Salvesen[26] have described a method for the determination of carbon monoxide in blood, the technic of which is exactly the same as that for the determination of oxygen (see above), except that after the gases are extracted the oxygen is absorbed in the apparatus by introducing alkaline pyrogallate solution. The carbon monoxide remains and is measured directly at atmospheric pressure.

Amino Acid Nitrogen.—The amino acid nitrogen received some comment in Chapter II, while the new Folin colorimetric method of estimating amino acid nitrogen was given in Chapter IX. Most of our knowledge regarding the amino acids of the blood and tissues has been obtained by the gasometric method of Van Slyke.[27] Several years ago Bock[28] made a careful study of the amino acid nitrogen content of normal and pathological human bloods with the Van Slyke method, slightly modified.

Blood Lipoids (fat, lecithin and cholesterol).—A more extensive discussion of cholesterol has already been given in Chapter VII.

In a study of the blood lipoids during fat assimilation, Bloor[29] has observed that (1) the total fatty acids increase in both plasma and corpuscles but the increase is generally more marked in the corpuscles; (2) lecithin increases greatly in the corpuscles, but only slightly in the plasma; (3) no definite change takes place in the quantity of cholesterol and (4) a fairly constant relationship exists between the total fatty acids and lecithin of the whole blood and corpuscles. From this Bloor suggests: (a) that the blood corpuscles take up the fat from the plasma and transform it into lecithin; (b) that most, if not all, of the absorbed fat is so transformed; and therefore (c) that lecithin is an intermediary step in the metabolism of the fats.

In normal bloods the lecithin content of the corpuscles is approximately double that of the plasma, while the cholesterol and total fatty acid values are almost always lower in the corpuscles than in the plasma. The value for lecithin in the corpuscles is generally about twice that of the cholesterol, while in the plasma their values are nearly equal. According to Bloor the ratio between these constituents is quite constant in normal blood (especially plasma) and remains so in most of the pathological samples, suggesting a definite relationship between these constituents, and making it probable that cholesterol (in the form of its esters) has a part in fat metabolism.

The most characteristic feature of pathological conditions is the increase of total fatty acids and fat both in plasma and corpuscles, and the decrease of lecithin in the

plasma. Since the fat is probably to be regarded as the inactive form of the body lipoids, the form in which they are stored and the lecithin as the first step in the utilization, an undue accumulation of fat or a notably decreased value for lecithin, probably indicates a diminished activity of the fat metabolism.

In severe diabetes[30] the blood lipoids are all greatly increased but the ratios between these constituents are practically normal. The fact that the cholesterol increases parallel with the fat in diabetic blood, even in severe lipemia, supports the view that probably cholesterol plays an important rôle in fat metabolism. Since cholesterol may be rather simply estimated, it affords a practical method of gauging the severity of diabetic lipemia (See Chapter VII). In mild diabetes the blood lipoids may be practically normal.

While there is no certain evidence that the abnormalities in the blood lipoids are responsible for anemia, the low values for cholesterol, which is an antihemolytic substance, and the high fat fraction, which may indicate the presence of abnormal amounts of hemolytic lipoids in the blood, are possible causative factors.

According to Bloor[31] the changes in the blood lipoids in severe nephritis are a high fat in the plasma and corpuscles and high lecithin in the corpuscles. These abnormalities are the same as are found in alimentary lipemia and may be regarded as the result of a retarded assimilation of fat in blood, due possibly to metabolic disturbances. As pointed out by Epstein (See Chapter VII) a marked lipemia and hypercholesterolemia is found in nephrosis.

Normally the *"total fat"* content of the blood plasma amounts to 0.6 to 0.7 per cent, but in severe diabetes figures as high as 26 per cent have been observed. In diabetic cases of ordinary severity, however, the figures amount to about 1.5 per cent. Nephritics frequently show a moderately increased fat although the figures (nephrosis excepted) rarely reach 1 per cent.

The normal figures for *lecithin* may be given in round numbers as 0.2 per cent for plasma, 0.3 per cent for whole blood and 0.4 per cent for corpuscles. In diabetes there is an increase in the lecithin of both the corpuscles and the plasma, although in severe lipemia it is more noticeable in the latter. In anemia the lecithin of the plasma in particular is lowered, while in nephritis there is a noteworthy increase in the corpuscles.

Cholesterol was made the topic of Chapter VII.

In 1914 Bloor described micro methods for the estimation of the blood lipoids, total fat, lecithin and cholesterol, the fat and lecithin being estimated nephelometrically and the cholesterol colorimetrically. These methods for total fat and lecithin (also cholesterol) are best given by Bloor[32] in a paper published in 1915. A year later the technic for the lecithin[30] was somewhat improved. Owing to various criticisms of the methods for fat and cholesterol, Bloor, Pelkan and Allen[33] have recently described a more refined technic of making these determinations.

Acetone Bodies

Owing to the importance which the acetone bodies hold in the acidosis, or more specifically the ketosis, occurring particularly in diabetes, the quantities of these substances—*acetone, aceto-acetic acid and β-hydroxybutyric acid*—present in normal and pathological human blood are of considerable interest. Acetone is a very diffusive substance and appears to be very readily and very rapidly eliminated by the kidney. For this reason the concentration in the urine is considerably greater than that in the blood, and likewise probably more significant. The amount of the β-hydroxybutyric acid present in both blood and urine is ordinarily in excess of the combined acetone-aceto-acetic acid fraction, often exceeding the latter by two or three times. According to Van Slyke and Fitz[34] the total acetone bodies of the blood normally amount to 1.3 to 2.6 mg. to 100 c.c. calculated as acetone, while in diabetes as much

as 350 mg. have been observed, although patients under ordinarily good control show 10 to 40 mg.

The Van Slyke technic of determining the acetone bodies in urine has been shown by Van Slyke and Fitz[34] to be directly applicable to blood after properly removing the proteins from the latter. Satisfactory results are obtained by precipitating the proteins at room temperature with the same mercuric sulfate solution utilized for precipitation of acetone.

Method.—(a) Whole Blood: Of whole blood 10 c.c. are diluted with about 100 c.c. of water (not more) in a 250 c.c. flask, and 20 c.c. of the 10 per cent mercuric sulfate are added. The solution is shaken for a moment, until the protein coagulates, and is then diluted with water up to the 250 c.c. mark. After 15 minutes or more it is filtered through a dry folded filter. If the first drops are cloudy, they are passed through a second time. The filtrate has a slight pink tinge, but the substance responsible for it does not precipitate when boiled with mercuric sulfate, nor otherwise interfere with the determination. (b) Plasma or Serum: 8 c.c. of oxalated plasma or serum are diluted in a 200 c.c. flask with 50 c.c. of water and 15 c.c. of the mercuric sulfate are added. The flask is shaken for a moment, until the fine precipitate which first forms has flocculated, and is then filled to the mark with water. After standing 15 minutes or longer the solution is filtered.

To determine the total acetone bodies together, 125 c.c. of the filtrate, equivalent to 5 c.c. of either blood or plasma, are treated exactly as the 25 c.c. of urine filtrate plus 100 c.c. of water in urine analyses (see page 194). One precaution should be observed. The precipitate should be filtered soon after the period of boiling is ended, otherwise several mg. of flocculent precipitate of indefinite origin may form and cause a plus error of appreciable magnitude in the results.

Phenols.—Benedict and Theis[35] have described a colorimetric method of estimating phenols in blood and have made observations on quite a large series of pathological cases. The values ranged from 1.9 to 8.0 mg. and averaged 4.7 mg. per 100 c.c. of blood. The figures for cases of hernia and sarcoma averaged higher than the other cases. Blood contains no conjugated phenols, but polyphenols appear to represent about one-third of the total phenols.

Calcium

The calcium content of human blood serum is normally very constant at somewhere between 9 and 11 mg. per 100 c.c., the figures averaging slightly higher in children than adults. It has been recognized for some time that the tetany following parathyroidectomy is associated with a decreased blood calcium and that the symptoms of tetany are relieved by calcium therapy. Similarly in infantile tetany the calcium falls, figures between 3.5 and 7.0 mg. being found where symptoms of tetany are present. The tetany is relieved by the persistent and constant administration of calcium, but with serum calcium figures of 7.5 mg. or more, symptoms do not appear.

Tetany appears to be the only condition constantly associated with a marked change in the blood calcium, although in some cases of advanced nephritis there may occasionally be a considerable reduction in the calcium (to 5 mg.) during the last days of life. As yet this drop in calcium has not been correlated with the so-called uremic symptoms.

Both Kramer and Tisdall,[36] and Clark[37] have described methods whereby the calcium is directly precipitated from the serum, plasma or whole blood without ashing or removal of the proteins. This direct precipitation has ob-

viated a very troublesome step in the determination, and not only simplified but probably increased its accuracy. The method, however, requires a highly refined chemical technic.

Since calcium is essentially a constituent of the plasma, and not the cells, the technic will be outlined for serum and plasma. Obviously, if plasma is to be used it must be citrated, not oxalated. For this purpose a saturated solution of sodium citrate is generally used. One c.c. (containing about 900 mg. sodium citrate), pH 7.4, is adequate for 10 c.c. of blood, although this is about the minimum quantity. Serum is preferable to plasma for the calcium estimation. In the method described the technic of Clark is followed.

Method.—Pipette 1 to 5 c.c. of serum or citrated plasma in a 50 c.c. conical centrifuge tube and, while rotating the tube, slowly add 3 per cent ammonium oxalate, equal in volume to one-half the amount of serum or plasma. Mix thoroughly and allow to stand over night. Rub down walls of the tube with a rubber policeman (washing the policeman with a small amount of distilled water), and centrifuge at moderate speed (1,800 R.P.M.) until clear; usually five minutes is ample time. Completely remove the supernatant liquid by means of a siphon (Tisdall has very recently pointed out that the supernatant liquid may be removed by careful decantation), stir up the precipitate with a fine stream of cold, distilled water, washing down the walls of the tube, using in all approximately 35 c.c. of water. Centrifuge immediately and completely siphon off the wash water. Dissolve the precipitate in 5 c.c. of approximately normal sulfuric acid,* heated to 75° C., and titrate with 0.01 N potassium permanganate† to the first faint pink. One c.c. of 0.01 N potassium permanganate is equivalent to 0.2 mg. of calcium.

Calculation.—The following simple formula may be used for the calculation: Titration in c.c. $\times$ 0.2 $\times \frac{100}{\text{cc. of serum employed}}$ = mg. of Ca to 100 c.c. of serum.

Magnesium.—The normal magnesium content of the blood of both adults and children (as Mg) generally falls between 2 and 3 mg. per 100 c.c. of plasma or serum, although with pathological bloods a somewhat wider range of 1 to 4 mg. may be found. A considerable number of different pathological conditions have been studied, but the findings differ very little from those found during health and do not appear to be characteristic of any pathological condition.

Simple colorimetric methods of determining magnesium in plasma or serum have recently been described by Briggs,[38] and by Denis.[39]

Sodium.—It has long been recognized that sodium is found chiefly in the body fluids, while potassium is a constituent principally of the cellular tissue. As might be expected, therefore, sodium is found chiefly in the blood plasma, and potassium in the corpuscles.

**Approximately Normal Sulfuric Acid.*—This may be prepared by adding 28 c.c. of pure conc. sulfuric acid to 970 c.c. of distilled water.

†*0.01 N Potassium Permanganate.*—It has been our custom to prepare this from a stock of 0.1 N solution by a 1 to 10 dilution, checking each preparation against 0.01 N oxalic acid solution. A very satisfactory oxalic acid standard may be prepared from ammonium oxalate. This is an anhydrous salt and may be obtained perfectly pure. To make a 0.1 N solution of oxalic acid weigh up 6.2045 gm. of ammonium oxalate, dissolve in about 500 c.c. of water, add 50 c.c. of pure conc. sulfuric acid, and make up to 1 liter. This is diluted to the 0.01 N strength as needed. To prepare the 0.1 N potassium permanganate, dissolve 3.162 gm. of potassium permanganate in about 900 c.c. of distilled water and allow to stand for a few days, then filter through glass wool. Check against the 0.1 oxalic acid and dilute to volume. The 0.01 N potassium permanganate is standardized, using the same apparatus as employed for the blood determination. Small portions of the 0.01 N oxalic acid (5 to 10 c.c.) are measured into the centrifuge tubes, subsequently to be used for the blood determination, heated to 75° C., and titrated, as a check on the standardization.

Clark[37] points out that in the titration of calcium oxalate obtained by direct precipitation the end-point is not as permanent as in the case of pure inorganic solutions and the amount of permanganate necessary for the blank is greater (especially true with whole blood). The presence of traces of organic matter accounts for the above conditions and in order to decide upon a reproducible end-point it is advisable for each operator to make a series of preliminary determinations. The blanks are determined from samples of whole blood and plasma which have been treated according to the regular procedures except that no oxalate is added. Clark found the following averages for blank determinations. (1) Whole blood = 0.10 c.c. of 0.01 N potassium permanganate; (2) plasma = 0.08 c.c. of 0.01 N potassium permanganate; and (3) sulfuric acid (5 c.c of N) = 0.05 c.c. of 0.01 N potassium permanganate.

The sodium content of human blood serum is very constant and averages about 335 mg. Na per 100 c.c., while for whole blood the figure is about 200 mg. Since there is very little sodium in the corpuscles the latter figure is without significance. Nothing of special importance is known regarding pathological variations in the sodium content of the blood.

A simple method for the direct quantitative determination of sodium in small amounts of serum has been described by Kramer and Tisdall.[40]

Potassium.—The potassium of normal human blood serum is a relatively constant quantity and amounts to close to 20 mg. K per 100 c.c. Although many observations have been made, there does not appear to be any significant variation in the potassium content of the serum in disease. The potassium content of whole blood depends in large measure upon the cell content, but appears to vary somewhere between 150 and 250 mg. per 100 c.c. in the normal human subject. In primary and secondary anemia the amount may obviously be very low.

A method of estimating potassium in animal fluids by means of sodium cobaltinitrite was described by Drushel in 1907 and was applied to spinal fluid in 1909 by Myers. The same technic was later applied to serum, plasma and whole blood by Myers and Short.[41] Kramer and Tisdall[42] have recently described a simple method whereby potassium may be precipitated with the cobalti-nitrite reagent directly from serum without ashing.

Iron.—As already pointed out, iron is present in hemoglobin to the extent of almost exactly one-third of one per cent, which would make the content of normal human blood about 50 mg. per 100 c.c. calculated as Fe. Pathologically, it varies directly with the hemoglobin content. Iron does not appear to be present normally in the plasma.

Wong[43] has recently described a simple and apparently very accurate method of estimating the iron content of blood.

Inorganic Phosphorus

As already pointed out (see page 22) the inorganic phosphorus of the blood (serum or plasma) of normal infants and children averages slightly above 5.0 mg. per 100 c.c., although in adults the average is somewhat lower, about 3.7 mg. In active rickets the inorganic phosphorus of the serum appears to be regularly reduced (below 3.7 mg.) sometimes to an extreme degree (2.0 mg). A low phosphorus may be found without much sign of rickets, but rickets seems to be the most important clinical disorder associated with low phosphorus. Although the test cannot be considered pathognomonic of rickets, it is of distinct value in early diagnosis.

It has recently been observed that following major fractures in adults there is a rise in the inorganic phosphorus content of the blood, in many instances to the level found in children. There appears to be a similar slight change in the calcium. This rise may take several days to two to four weeks. After union the phosphorus gradually falls. As a rule cases with nonunion do not show this reaction in the blood phosphorus following fracture.

In cases of advanced chronic nephritis with more or less marked nitrogen retention there is generally a considerable increase in the inorganic phosphorus content of the blood, figures as high as 15 mg. and even more having been observed. The phosphorus seems to bear some relation to the acidosis and to be an unfavorable prognostic sign, although the full significance of this relationship is not understood at the present time.

A colorimetric method of estimating the inorganic phosphorus of blood

was first described by Bell and Doisy.[44] The alkaline blue color which they employed for color comparison fades rather rapidly, and for this reason Briggs[45] modified the method so as to carry out the determination in an acid solution. In this modification he utilized the "stabilizing" action of sodium sulfite first employed in this connection by Benedict and Theis[35] for the estimation of phenols in blood. Although the color does not fade with this procedure, it is comparatively weak.

Benedict[46] (private communication) has recently developed a technic which yields a deep nonfading color that may be very accurately matched. This is the method described below.

Method.—Transfer 2 c.c. of serum or plasma to a test tube, and add 4 c.c. (2 volumes) of water and 4 c.c. (2 volumes) of 20 per cent trichloracetic acid. Shake vigorously and after 10 minutes transfer the contents to a dry ashless filter. Filtration can conveniently be made into a test tube.

Transfer 3 c.c. of the filtrate (equivalent of 0.6 c.c. of serum) to a sugar tube and add 5 c.c. of water. To a similar tube add 3 c.c. of a phosphate standard* containing 0.025 mg. of phosphorus and dilute with 5 c.c. of water. Now add to both tubes 1 c.c. of Benedict's hydroquinone-bisulfite reagent† and 1 c.c. of his molybdic acid reagent.†† Loosely stopper the tubes and heat in a boiling water bath for 10 minutes. Cool and compare in the colorimeter.

Calculation.—The following formula may be employed for the calculation:

$$\frac{15}{R} \times 0.025 \times \frac{100}{0.6} = \text{mg. of P in 100 c.c. of serum or plasma.}$$

Owing to the heating, whole blood cannot be used for the estimation of the inorganic phosphorus, but may be employed when it is desired to estimate the hydrolyzable plus the inorganic phosphorus. In this case less filtrate should be used and heating in the water bath must be continued until hydrolysis is complete and the maximum color is obtained, about one hour.

Sulfates.—The inorganic sulfates of human blood, calculated as S, amount to from 0.5 to 1.0 mg. per 100 c.c. In nephritics with nitrogen retention there is also found a retention of inorganic sulfates, figures as high as 16 mg. having been obtained.[47]

The only method so far described for the estimation of sulfates in blood is the nephelometric method of Denis.[48]

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**Standard Phosphate Solution.*—A standard solution containing 0.025 mg. of P per 3 c.c. of solution may be prepared by dissolving 0.1100 gm. of highest purity dry KH_2PO_4 in water and making up to 300 c.c. It should be preserved with chloroform.

†Benedict's Hydroquinone-Bisulfite Reagent.—This reagent may be prepared by dissolving 30 gm. of sodium bisulfite and 1 gm. hydroquinone in water and making up to 200 c.c.

††Benedict's Molybdic Acid Reagent.—Dissolve 20 gm. of molybdic acid (Eimer and Amend 99.9 per cent pure) in 25 c.c. of 20 per cent sodium hydroxide with warming. Dilute to 200 c.c. and filter if necessary. Transfer to a liter flask and add with constant agitation under cold running water an equal volume (about 200 c.c.) of concentrated sulfuric acid.

The reagent possesses a fairly deep blue color, but on dilution with the unknown or standard, previous to heating, the color disappears.

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CHAPTER XI

CHEMICAL BLOOD ANALYSES, THEIR CLINICAL USE AND INTERPRETATION

A NUMBER of practical questions may arise in connection with the chemical examination of the blood for diagnostic purposes, such as how should the specimen be taken, how may the results be recorded and reported, what information may one expect from such an examination and what analyses should be requested in having an examination made.

For the ordinary chemical examination of blood 10 to 20 c.c. are required, and can best be drawn directly into a bottle containing as the anticoagulant 2 to 3 drops of 20 per cent potassium oxalate (dried in the bottle). If possible the specimen should be taken in the morning before breakfast. In case the specimen is taken outside the hospital or laboratory, provision should be made for getting the specimen to the laboratory at once. When refrigerated, blood may be kept suitable for some determinations for several days. Analyses on specimens *sent by mail* (without refrigeration) and not delivered and analyzed within 24 hours are very frequently *valueless.*

Calcium can best be determined in the serum. It cannot be estimated in oxalated blood.

A report blank which we employ, and which is fairly well suited to clinical and scientific purposes is given on page 182.

In this report blank the determinations which have been found to be of special practical usefulness have been starred. These determinations are obviously not of equal value in all cases, and the biochemist making the blood analysis will be greatly aided if he possesses some information regarding the probable diagnosis, the clinical symptoms or has specific but intelligent requests for individual determinations. This is particularly important when the material at hand is limited.

Renal Diabetes

True renal diabetes is a condition of glycosuria without hyperglycemia. A knowledge of the blood sugar is therefore necessary for a correct diagnosis of this rather uncommon condition.

Diabetes Mellitus

In diabetes mellitus the blood sugar is obviously the determination of first interest, although in advanced cases the CO_2-combining power may assume greater importance. With moderately increased figures for the blood sugar, the CO_2 should always be estimated. Except in the terminal stages with coma, the acidosis appears to be quite completely compensated. Con-

sequently the CO_2-combining power furnishes very reliable information as to the degree and severity of the acidosis. Insulin appears to be a specific therapeutic agent for the treatment of severe diabetic acidosis.

Gout

In gout interest centers about the uric acid; still, a knowledge of the urea nitrogen content is necessary in order to rule out as far as possible a uric acid retention of purely nephritic origin. In the absence of renal involvement the blood uric acid is not increased in infectious arthritis.

Nephritis

In nephritis, particularly of the interstitial type, the first sign of impairment in renal function is, as a rule, an increase in the uric acid. When the symptoms become fairly definite, however, varying degrees of urea retention are observed. Figures for urea N remaining above 20 mg. per 100 c.c. are very good evidence of impaired renal function. Creatinine is the last nitrogenous waste product to be retained, and figures over 5 mg. give a very bad prognosis. With high figures for urea, creatinine should always be estimated.

Acute nephritis is sometimes accompanied by severe *acidosis,* while most cases of chronic nephritis with marked nitrogen retention show an acidosis, sufficient in some cases to cause death. The CO_2-combining power should always be determined when any acidosis symptoms are present, particularly with urea nitrogen figures above 30 mg. The acidosis here is apparently not always completely compensated, so that there may be a change in the pH value of the blood with only a moderate reduction in the blood bicarbonate. The estimation of the CO_2 is, however, the best clinical test at present available. Just what relation the retention of phosphates bears to the acidosis is not clear, still the rise in the inorganic phosphorus of the blood appears to furnish information of some little prognostic value. In cases of acidosis the administration of sodium bicarbonate can be fairly accurately gauged when a knowledge of the CO_2 is at hand.

It has long been customary to restrict the chloride content of the diet in cases of nephritis with *edema.* This can be done much more intelligently with a knowledge of the chloride content of the blood, especially since edema is not necessarily dependent upon salt retention.

In nephrosis the one noteworthy feature is the hypercholesterolemia.

Mercury Bichloride Poisoning

Poisoning with mercuric bichloride frequently results in complete suppression of urine. This obviously brings about an immediate and rapid rise of the nitrogenous waste products in the blood. For this reason a high creatinine here does not furnish the same grave prognosis that it does in chronic interstitial nephritis where the accumulation has been slow. Still a knowledge of these blood findings will generally forecast the ultimate outcome.

Report on Chemical Examination of Blood

Clinic No..... Serial No.....

Chart No..... Date of Specimen.....

Name.....Ward.....Date of Report.....Age.....Sex .....

Volume obtained .....c.c.

per cent

Total Solids .....

*Hemoglobin .....

Total Nitrogen .....

Nonprotein Nitrogenous Constituents

mg. per 100 c.c.

Nonprotein N .....

*Uric Acid .....

*Urea N .....

*Creatinine .....

Creatine .....

Amino Acid N .....

Miscellaneous Constituents

per cent

*Sugar .....

Diastatic Activity .....

Cholesterol .....

Fat .....

Acetone Bodies .....

*Chlorides as NaCl .....

mg. to 100 c.c.

Calcium as Ca .....

Inorganic Phosphates as P .....

*CO_2-Combining Power.....c.c. CO_2 per 100 c.c. plasma

Hydrogen Ion Concen. (pH).....

Oxygen .....

Remarks:

.....

.....

.....

Lab. No.....

Urologic Conditions

Preoperative information on the chemistry of the blood should be secured in all surgical conditions of the kidneys, bladder or prostate. The information is of more value in prostatic than in other urologic lesions. The blood urea in particular furnishes an excellent preoperative prognosis as far as the kidneys are concerned. With urea nitrogen figures of 20 mg. or less, the renal factor can quite safely be eliminated. With urea retention, however, the creatinine or CO_2-combining power may be more significant. Patients presenting urea nitrogen figures over 25 mg. should be operated on with caution, and best after a period of preliminary treatment directed to relieve the nitrogen retention. This applies particularly to any lesion inhibiting the output of urine.

Eclampsia

In true eclampsia the nonprotein nitrogen is moderately increased, but the urea nitrogen is at the low normal limit or decreased, constituting 15 to 40 per cent of the nonprotein nitrogen instead of the normal 50 per cent. As a rule the uric acid is definitely increased, due apparently to a slight impairment of renal function. Judging from the CO_2-combining power there is a moderate or severe acidosis in all cases. Some cases show high blood chlorides and marked edema, others a slight but definite hyperglycemia. Toxemias do occur in which nitrogen retention exists, but it seems probable that in most cases this is due to a true nephritis.

Pneumonia

Severe pneumonia is often accompanied by marked nitrogen retention and pronounced acidosis, and, sometimes in the terminal stages of the disease, by a marked decrease in the oxygen saturation of the blood.

Malignancy

Malignancies in the terminal stages often show considerable nitrogen retention and acidosis.

Intestinal Obstruction

Intestinal obstruction may also be accompanied by marked nitrogen retention, while in cases with gastric and duodenal ulcer there is often a definite increase in the urea nitrogen.

Infantile Tetany

Since infantile tetany is apparently dependent upon a decrease in the calcium content of the blood and is relieved by the administration of calcium, a knowledge of the blood calcium is necessary for a correct diagnosis and an intelligent therapy.

Rickets

In active rickets the inorganic phosphorus of the serum appears to be regularly reduced, sometimes to an extreme degree. A low phosphorus

Report on Sugar Tolerance Test

Chart No..... Serial No.....

Clinic No..... Date of Specimens..... Date of Report.....

Name..... Ward..... Weight..... Age..... Sex.....

Clinical Diagnosis..... Carbohydrate Given..... Gms..... Time..... Fluid.....c.c.

Time	Blood Sugar	Urine			Blood Sugar	Hour - Blood——— Urine - - - - - -						Urine Sugar
		Volume	Sugar	Sugar		1st	2nd	3rd	4th	5th	6th	
	per cent	c.c.	per cent	mg.	per cent							mg.
					0.36							1400
					0.32							1200
					0.28							1000
					0.24							800
					0.20							600
					0.16							400
					0.12							200
		Total			0.08							0

Remarks.....

..... Lab. No.....

may be found without much sign of rickets, but rickets seems to be the most important clinical disorder associated with a low phosphorus, and although the test cannot be considered pathognomonic of rickets, it is of distinct value in early diagnosis. Cod liver oil and ultra violet light, the two therapeutic agents most used in the treatment of rickets, both raise the blood phosphorus.

Diarrheal Acidoses of Infancy

As might be expected, the diarrheal acidoses of infancy are accompanied by a marked lowering in the CO_2-combining power.

Pancreatic Disease

In pancreatic disease the blood sugar is generally slightly increased, while the increase in the diastatic activity is quite marked. It is of interest in this connection that hyperglycemia is frequently found in cases of cholelithiasis especially those with cholecystitis.

Endocrine Disorders

In the hyperendocrine disorders, particularly *hyperthyroidism,* an increase in the blood sugar is often noted when the blood is taken in the middle of the day, although in the morning the sugar content may be quite normal. In hypo-conditions, such as *Addison's disease,* the blood sugar is normal or subnormal. Disturbances in carbohydrate tolerance can best be brought out by a glucose tolerance test, (see page 88) giving 1.75 gm. glucose per kg. body weight, and following the blood sugar until the type of curve is evident. The form of report blank we employ is given on the opposite page.

The blood determinations specially indicated in various conditions may be summed up in tabular form somewhat as follows:

DISORDER	CHEMICAL BLOOD TESTS INDICATED
Renal Diabetes	Sugar
Mild Diabetes	Sugar, CO_2
Severe Diabetes	Sugar, CO_2; some cases diastatic activity, cholesterol and urea
Gout	Uric acid, urea
Early Interstitial Nephritis	Uric acid, urea
Late Interstitial Nephritis	Urea, creatinine, CO_2, inorganic phosphorus
Acute Nephritis	Urca, creatinine, CO_2, chlorides
Nephritis (Parenchymatous) with Edema	Urca, chlorides
Nephrosis	Cholesterol, serum proteins
Mercury Bichloride Poisoning	Urca, creatinine, CO_2
Urologic Conditions	Urea, CO_2; severe cases creatinine
Eclampsia	Nonprotein N, urea, CO_2, uric acid, chlorides
Pneumonia	Urea, CO_2, oxygen content
Malignancy	Urea, CO_2
Intestinal Obstruction	Urea, creatinine
Infantile Tetany	Calcium
Rickets	Inorganic phosphorus, calcium
Diarrheal Acidoses of Infancy	CO_2, inorganic phosphorus
Pancreatic Disease	Sugar, diastatic activity
Endocrine Disorders	Glucose tolerance test

CHAPTER XII

QUANTITATIVE MICRO-METHODS OF URINE ANALYSIS

ALTHOUGH the practical information which can now be obtained from the blood would appear to greatly exceed the value of these tests when carried out on the urine, nevertheless an analysis of the urine often gives added information. Ambard[1] in his very interesting and instructive book on "Physiologie normale et pathologique des reins," has endeavored to show that the information obtainable by the comparative study of the blood and urine is much greater than that obtained from either the blood or urine alone (see Chapter II, page 37).

Blood analyses are of little help in comparing the activity of the two kidneys, since the blood gives only their activity collectively. Nevertheless since blood analyses indicate the condition of both kidneys, they are of value in determining the operability of patients with unilateral renal lesions. In such cases, the blood findings are usually within normal limits. However, in lesions necessitating nephrectomy, the toxemia produced by the renal infection sometimes temporarily impairs the function of the other kidney, so that the blood urea nitrogen may be 30 mg. or more. When the diseased kidney has been removed, the urea drops to normal. To ascertain the activity of each kidney it is necessary to compare their excretory power individually. This may be done with the aid of the phthalein test or with such determinations as total nitrogen and chlorides, employing specimens collected from the catheterized ureters.

REPORT ON REACTION TO RENAL TEST MEAL

Clinic No..... Serial No.....
Chart No..... Date of Specimens.....
Name..... Ward.....Date of Report.....Age.....Sex.....
Diet .....

Time	Volume c. c.	Sp. Gr.	Sodium Chloride		Nitrogen	
			Per Cent	Grams	Per Cent	Grams
8 — 10	.....	.....	.....	.....	.....	.....
10 — 12	.....	.....	.....	.....	.....	.....
12 — 2	.....	.....	.....	.....	.....	.....
2 — 4	.....	.....	.....	.....	.....	.....
4 — 6	.....	.....	.....	.....	.....	.....
6 — 8	.....	.....	.....	.....	.....	.....
Total Day { 8 a. m. / 8 p. m.	.....	.....	.....	.....	.....	.....
Night { 8 p. m. / 8 a. m.	.....	.....	.....	.....	.....	.....
Total 24 hrs.						

1. Void urine at 8 a. m. and discard.
2. Continue regular diet with exception that NO FOOD OR FLUID IS TO BE TAKEN AFTER SUPPER until 8 a. m. next morning.
3. Collect specimens as per schedule, completing final specimen by voiding at 8 a. m.

Lab. No.....

If one utilizes the renal test meal suggested by Mosenthal,[2] collecting two-hourly day and a night specimen of urine, or simply compares the day and night urines after a regular meal, as a means of testing the kidney function, nitrogen and chloride estimations are necessary. The simple methods described below are especially well adapted to such work.

The report blank shown on the opposite page may be conveniently used for the Mosenthal renal function test.

For most cases it is sufficient to measure the volume and specific gravity of the two hourly day specimens, and estimate the nitrogen and chlorides on only the day and night specimens. The application of this and other tests in nephritis has been most excellently discussed by Mosenthal[2] in his chapter on Metabolism in Nephritis in "Endocrinology and Metabolism."

Estimation of Chlorides

Harvey[3] has described a slight modification of the Volhard method of chloride estimation in urine which is rapid and reasonably accurate.

Method.—Five c.c. of urine are pipetted into a small porcelain evaporating dish and diluted with about 20 c.c. of distilled water. The chlorides are now precipitated with exactly 10 c.c. of the standard silver nitrate solution* and about 2 c.c. of the acidified indicator† added. Standard ammonium thiocyanate solution†† is then run in from a burette until the first trace of yellow shows throughout the mixture. By subtracting the number of c.c. of thiocyanate thus employed from ten and multiplying by 0.01, the amount of sodium chloride in 5 c.c. of urine is obtained in gm. From this the percentage of chlorides is calculated as NaCl or the output for the 24 hours may be computed.

Micro-Kjeldahl for Total Nitrogen

The method described[4] is a slight simplification of the method of Folin and Farmer.[5] For the determination an amount of urine sufficient to contain between 0.5 and 1.0 mg. of nitrogen is required. This is usually obtained with a 1 to 10 dilution of urine.

Method.—One c.c. of urine is taken with an Ostwald-Folin pipette and diluted to 10 c.c. with distilled water in a dry test tube with a similar 9 c.c. pipette. After thorough mixing, 1 c.c. of the diluted urine is pipetted into a thin glass test tube (about 18x150 mm.), 0.1 c.c. of concentrated sulfuric acid (special nitrogen free), 50-100 mg. (knife point full) of potassium sulfate and 2 drops of 10 per cent copper sulfate added. The tube is now boiled by hand over a microburner flame with continued shaking until fumes appear (1 to 2 min.) and then for two or three minutes until the solution begins to clear. At this point 1 drop of hydrogen peroxide§ is added, whereupon the solution turns a clear blue almost without further heating. As a means of absorbing the fumes which appear during the latter stages

*Prepared by dissolving 29.06 gm. of pure silver nitrate in, and making up to one liter with distilled water. Each c.c. of such a solution is equivalent to 0.01 gm. of sodium chloride.

†The acidified indicator is prepared by dissolving 100 gm. of crystalline ferric ammonium sulfate in 100 c.c. of 25 per cent nitric acid.

††The standard ammonium thiocyanate is prepared by dissolving about 13 gm. in 800 c.c. of distilled water and then determining, according to the method described for the titration, how much of the solution is required to completely precipitate 10 c.c. of the silver solution. From this one may calculate how much water must be added to the remainder of the thiocyanate solution to make it exactly equivalent to the standard silver solution.

§Wong[6] has recently suggested the use of potassium persulfate as an oxidizing agent. In this case it is substituted for both the potassium sulfate and the hydrogen peroxide. Except for the omission of the potassium sulfate the procedure is carried out as outlined, but 2 drops of a saturated solution of potassium persulfate are substituted for the hydrogen peroxide. It appears to be the equal but not the superior of hydrogen peroxide.

of the oxidation, a piece of wet filter paper may be placed in the mouth of the test tube. When digestion is complete the tube is allowed to cool for a minute and then washed into an accurate 100 c.c. graduated cylinder with about 35 c.c. of distilled water.

Five c.c. of the standard nitrogen solution containing 1 mg. of nitrogen* are pipetted into a 100 c.c. volumetric flask and about 50 c.c. of distilled water added. Ten c.c. of the modified Nessler's solution† are diluted just previous to use to 50 c.c. with distilled water. About 20-25 c.c. of the diluted Nessler's solution are now added to the standard, after which it is made up to volume with distilled water and mixed. The standard cell or cup of the colorimeter is now filled and set at the 15 mm. mark. To the unknown in the 100 c.c. cylinder a similar amount of diluted Nessler's is at once added and the solution diluted until approximately the same intensity of color is reached as the standard. Since the unknown still contains considerable sulfuric acid, it is essential that the Nessler's should be dumped in all at once while rotating the solution in the cylinder to prevent the development of turbidity.

Calculation.—For the calculation the following formula may be used:

$$\frac{15}{R} \times 0.001 \times \frac{D}{100} \times \frac{\text{24 hr. Vol. in c.c.}}{0.1} = \text{Nitrogen output in grams,}$$

in which R represents the reading of the unknown, 0.001 the strength of the standard, D the dilution of the unknown and 0.1 the amount of urine employed.

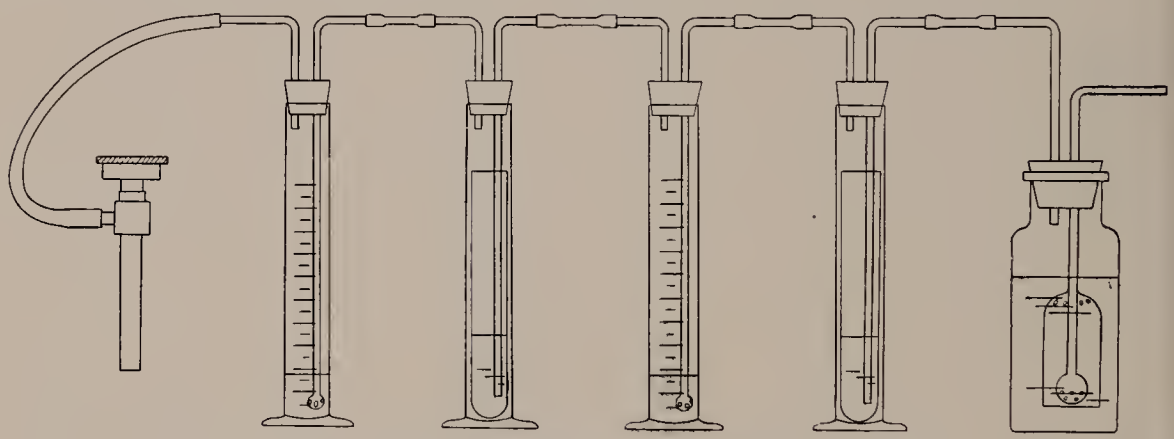

Fig. 23.—Aeration apparatus for urea and ammonia nitrogen estimation.

For example, with a dilution of 75, a reading of 10 and a 24 hour volume of 1000 c.c. the formula would work out as follows: $\frac{15}{10} \times 0.001 \times \frac{75}{100} \times \frac{1000}{0.1} = 11.25$ gms. nitrogen in the 24 hour urine.

Estimation of Urea

Although some of the older "macro" methods probably give more accurate results for the estimation of urea in urine, still very good figures may be obtained by using the same apparatus as is employed in the estimation of urea in blood, illustrated in Fig. 23 above.

Ordinarily 0.1 c.c. of urine is about the right amount to employ for the estimation. (In case the total nitrogen estimation is being made one can use 1 c.c. of the same diluted urine.) One c.c. of urine is taken with an Ostwald-Folin pipette and diluted to 10 c.c. with distilled water in a dry test tube with a similar 9 c.c. pipette. After thorough mixing, 1 c.c. of the diluted urine is pipetted with the Ostwald-Folin pipette into a test tube about 150 mm. in length and of diameter (20 mm.) such that it will readily slip into a 100 c.c. cylinder.

*A standard solution containing 1 mg. of N per 5 c.c. of solution may be prepared by dissolving 0.944 gm. ammonium sulfate or 0.764 gm. ammonium chloride of the highest purity in distilled water and making up to 1000 c.c.

†See page 215.

To the dilute urine is now added 1 c.c. of a 5 per cent jack bean urease solution,* and the tube (or tubes) incubated in a beaker of water at 50°C. for 15 minutes.

Into a 100 c.c. graduated cylinder without lip are added 15 c.c. of distilled water and 2 c.c. of 0.1 N hydrochloric acid or 2 to 3 drops of the 10 per cent solution. This is now closed with a two-hole stopper having a glass tube passing nearly to the bottom of the cylinder. The tube is sealed at the lower end but contains a number of small holes to aid in the complete absorption of the ammonia. The apparatus to connect the pair of cylinders is adjusted, and to the test tube containing the urease digested urine are added 1 to 2 c.c. of amyl alcohol (or 4 to 5 drops of pure caprylic alcohol) and then about 4 to 5 c.c. of saturated sodium (or even better, potassium) carbonate solution, after which the tube is placed in the ungraduated cylinder and the stopper quickly inserted. The cylinder containing the digested mixture is now connected with a suction pump and air slowly allowed to pass through the apparatus, the speed being increased so that at the end of two minutes the air current is as rapid as the apparatus will stand. A series of a half dozen or more tubes may conveniently be set up in this way. Aeration is complete in about 30 minutes and the apparatus is then disconnected.

Into a volumetric flask of 100 c.c. capacity are pipetted 5 c.c. of ammonium sulfate solution† containing 1 mg. of nitrogen and 50 to 60 c.c. of distilled water are added. Sufficient of the modified Nessler's solution†† for the standard and the unknown is diluted about five times with distilled water, and of this about 15 c.c. added to the standard solution which is made up to the mark with water. At the same time about 15 c.c. of the freshly diluted Nessler's solution are added to the unknown and the volume made up to 50 c.c. in the graduate, unless a high content of urea nitrogen is indicated, in which case a dilution of 75 or 100 may be needed to make the color of the unknown approximately the same intensity as the standard. This can best be set at the 15 mm. mark on the colorimeter.

Calculation.—To calculate the output of urea nitrogen the same formula is employed as for the total nitrogen, viz.: $\frac{15}{R} \times 0.001 \times \frac{D}{100} \times \frac{\text{24 hr. Vol. in c.c.}}{0.1} = \text{Urea N output}$ in grams.

Since urine always contains preformed ammonia it is necessary to correct for the ammonia nitrogen in any accurate estimation of urea.

If the figures are desired in terms of urea instead of urea nitrogen, multiply by the factor 2.14 after deducting the amount of the ammonia nitrogen.

Estimation of Ammonia

Aeration Method.—When the ammonia estimation is carried out along with an estimation of urea, the aeration procedure may be conveniently employed.

Method.—With normal urine 2 c.c. will usually contain the right amount of ammonia nitrogen, although dilute urines may require as much as 5 c.c., and with diabetic urines 1 c.c. may even be too much. The apparatus is arranged as for the urea estimation. To 2 c.c. of urine in the same size tube as employed for the urea add 1 to 2 c.c. of amyl alcohol (or 2 to 3 drops of pure caprylic alcohol) to prevent foaming and 4 c.c. of saturated sodium carbonate. Aeration and Nesslerization are carried out just as for the urea. The calculation is the same as with the method described below.

Permutit Method.—Folin and Bell[7] have recently suggested an extremely simple procedure of estimating ammonia in urine with the aid of permutit (a synthetic zeolite powder which has a great adsorptive power for ammonia).

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*Concentrated dry preparations of jack bean urease may be obtained on the market (Arlington, Squibb), prepared according to the Van Slyke formula. These have the activating phosphate already added. The solution is made up just previous to use. For Folin and Wu's method of preparing urease see page 224.

†See total nitrogen, page 216.

††See page 215.

Method.—Transfer about 2 gm. of the powder to a 100 c.c. volumetric flask, add 5 c.c. of water and then with an Ostwald-Folin pipette introduce 1 or 2 c.c. of urine. Rinse down the added urine with the aid of a little water (1 to 5 c.c.), and shake gently but continuously for 5 minutes. Rinse the powder to the bottom of the flask by the addition of water (25 to 40 c.c.) and decant. Add water once more and decant. Add a little water to the powder, introduce 5 c.c. of 10 per cent sodium hydroxide, mix, and then add more water until the flask is about three-fourths full. Shake for a few seconds and then add 10 c.c. of diluted Nessler's solution. Mix and let stand for about ten minutes, or longer. Make up to volume, mix and compare with the standard, setting the cell or cup at the 15 mm. mark.

The standard is the same as that employed for the total nitrogen and urea, that is, ammonium sulfate made up to contain 1 mg. to 5 c.c. Five c.c. of the standard are pipetted into a 100 c.c. volumetric flask, about 60 to 70 c.c. of water added and then 5 c.c. of 10 per cent sodium hydroxide and 10 c.c. of the diluted Nessler's as in the unknown, and finally diluted to 100 c.c.

Calculation.—For the calculation the following formula may be used:

$$\frac{15}{R} \times 0.001 \times \frac{D}{100} \times \frac{\text{24 hr. Vol. in c.c.}}{2} = \text{Ammonia N output in 24 hrs.}$$

Estimation of Creatinine

The original Folin[8] method gives perfectly satisfactory results. With the method as described, the quantity of urine and reagents have been reduced to one-fifth the quantity originally employed in 1904.

Method.—Fill the 8x10 mm. cell of the Bock-Benedict colorimeter with 0.5 N potassium bichromate, and set the cell to read 8 mm. (If the Duboscq or Klett instrument is employed, the left hand cup is set at the 8 mm. mark.) Pipette 2 c.c. of urine (1 c.c. may be sufficient if the urine is very concentrated, while in the case of dilute urine 3 c.c. may be required) into a 100 c.c. volumetric flask and add 3 c.c. of saturated picric acid solution and 1 c.c. of 10 per cent sodium hydroxide. Shake thoroughly and allow the mixture to stand for five minutes. At the end of this time the contents of the flask are diluted to the 100 c.c. mark with tap water, thoroughly mixed and a portion poured into the empty cylinder of the colorimeter. A number of readings are taken immediately.

Calculation.—Folin originally obtained the factor of 8.1 mm. for pure creatinine under conditions similar to the above. This being the case the following formula may be used:

$$\frac{8.1}{R} \times 0.002 \times \frac{\text{24 hr. Vol. in c.c.}}{\text{Urine used in c.c.}} = \text{grams of creatinine excreted in 24 hours.}$$

For example with a reading of 8.1 mm., 2 c.c. of urine employed and a 24 hour volume of 1000 c.c. the formula would give $\frac{8.1}{8.1} \times 0.002 \times \frac{1000}{2} = 1.00$ gm. of creatinine.

It is best to adjust the amount of urine, by employing 1, 2 or 3 c.c., to give readings between 6 and 10 mm. on the colorimeter, as lower or higher readings give less accurate results.

Estimation of Creatine

Method.—For the conversion of creatine to creatinine the method of Benedict[9] may conveniently be employed. Two c.c. of urine are taken, as in the determination of creatinine, placed in a medium sized test tube, 2 c.c. of N hydrochloric acid (concentrated hydrochloric acid diluted 1 to 10) and a very little powdered metallic lead added. The contents of the tube are now carefully boiled nearly to dryness over a free flame, then washed with as small an amount of water as possible through a very small cotton or glass wool filter into

a 100 c.c. volumetric flask or graduate. This is done to remove the metallic lead which also reacts with the picric acid and alkali. The color is now developed in the same way as for the creatinine except that 2 c.c. of alkali are employed. The result obtained represents the total creatinine. The difference between the preformed and the total creatinine gives the creatine in terms of creatinine. By multiplying this value by 1.16 the weight of the creatine may be obtained.

Estimation of Uric Acid

Benedict and Franke[10] have recently described a very simple colorimetric method whereby uric acid may be estimated directly in the urine without the previous separation of the uric acid. This has been made possible by the use of Benedict's new arsenic phosphoric tungstic acid reagent, first employed on blood filtrates (see page 61). This new reagent under the conditions used is highly specific for uric acid. With the new procedure the estimation of uric acid in urine is quite as simple as the creatinine determination.

Method.—The urine is so diluted that 10 c.c. will contain between 0.15 and 0.30 mg. of uric acid. (Usually a dilution of 1 to 20 is satisfactory.) 10 c.c. of the diluted urine are measured into a 50 c.c. volumetric flask, 5 c.c. of the 5 per cent sodium cyanide* solution are added from a burette, followed by 1 c.c. of the arsenophosphotungstic acid reagent.† The contents of the flask are mixed by gentle shaking, and at the end of 5 minutes diluted to the 50 c.c. mark with distilled water and mixed. This blue solution is then compared in a colorimeter with a simultaneously prepared solution obtained by treating 10 c.c. of the standard uric acid solution†† (0.2 mg. of uric acid) in a 50 c.c. flask with 5 c.c. of the sodium cyanide solution, 1 c.c. of the reagent, and diluting to the mark at the end of 5 minutes.

Calculation.—The calculation of results is very simple. The reading of the standard (15 or 20 mm.) divided by the reading of the unknown, and the result multiplied by 0.2, gives the mg. of uric acid contained in the 10 c.c. of the diluted urine used in the unknown.

Calculation of the uric acid content of the 24-hour specimen may be made with the following formula:

$$\frac{15}{R} \times 0.0002 \times \frac{\text{24 hr. vol. in c.c.}}{\text{c.c. of urine employed}} = \text{24 hr. output of uric acid in grams, in which}$$

R stands for the reading of the unknown.

**Sodium Cyanide.*—A 5 per cent solution of sodium cyanide, which should be prepared fresh once in about 2 months, is used.

†Benedict's New Uric Acid Reagent.—The reagent employed is the one used in the new procedure for the direct determination of uric acid in blood, and is prepared as follows: 100 gm. of pure sodium tungstate are placed in a liter Pyrex flask and dissolved in about 600 c.c. of water. 50 gm. of pure arsenic acid (As_2O_5) are now added, followed by 25 c.c. of 85 per cent phosphoric acid and 20 c.c. of concentrated hydrochloric acid. The mixture is boiled for about 20 minutes, cooled, and diluted to 1 liter. The reagent appears to keep indefinitely.

††Benedict's Dilute Uric Acid Standard.—A standard solution of uric acid, acidified with hydrochloric acid, containing 0.2 mg. of uric acid in 10 c.c., is employed. This solution may be readily prepared by dilution of the phosphate standard uric acid solution of Benedict, as follows: 50 c.c. of the phosphate standard solution (containing 10 mg. of uric acid) are measured into a 500 c.c. volumetric flask and diluted to about 400 c.c. with distilled water. 25 c.c. of dilute hydrochloric acid (made by diluting 1 volume of the concentrated acid with 9 volumes of water) are added, and the solution is diluted to 500 c.c. and mixed. This dilute standard solution should be prepared fresh from the phosphate standard every 10 days to 2 weeks.

Benedict's Phosphate Standard is prepared as follows: Dissolve 4.5 gm. of pure crystalline hydrogen disodium phosphate and 0.5 gm. of dihydrogen sodium phosphate in about 200 c.c. of hot water. Pour this warm, clear solution on 100 mg. of uric acid suspended in a few c.c. of water in a 500 c.c. beaker. Agitate with a stirring rod until the uric acid is *completely* dissolved. Now add at once exactly 0.7 c.c. glacial acetic acid. Pour into a 500 c.c. volumetric flask, cool, make up to 500 c.c. mark with distilled water, mix and add 5 c.c. of chloroform as preservative. Fifty c.c. of this solution contain 10 mg. of uric acid. The solution should be freshly prepared every 4 to 8 weeks.

For example, with a 24-hour volume of 800 c.c., 0.5 c.c. of urine used, and a reading of 12, the formula would work out as follows:

$$\frac{15}{12} \times 0.0002 \times \frac{800}{0.5} = 0.40 \text{ gm. uric acid.}$$

Some explanatory remarks in connection with the technic may be of value. The proportional depth of color for uric acid concentrations varying between 0.15 and 0.30 mg. is almost absolutely exact under the conditions indicated, when 0.2 mg. of uric acid is used as the standard. Outside of this range the results are not quite satisfactory, and cannot be materially improved by greater or less dilution of the unknown. It is essential that the volume of the unknown and of the standard be the same during the period of the reaction. This volume may be 15 c.c., instead of the 10 c.c. indicated, if desired for any special reason, but in such cases the standard must be diluted to the same volume before addition of the reagents.

Micro-Method of Estimating Sugar in Urine

An estimation of the sugar content of urine, sufficiently accurate for clinical purposes, can be made with Benedict's* volumetric method,[11] employing a large test tube and a 1 or 2 c.c. Mohr pipette for the titration. This is a method which we have used for teaching and routine clinical purposes for a number of years.

Method.—Five c.c. of Benedict's volumetric solution are pipetted into a test tube (25 x 150 mm.) and 1 to 2 gm. of sodium carbonate added. (If preferred a 25 c.c. Erlenmeyer flask may be employed, instead of the test tube, and the flask placed over a wire gauze.) The solution is now brought to a vigorous boil with continued gentle agitation, the tube being held in the left hand, employing a folded paper as a test tube holder. With the right hand the urine is run in, a drop at a time, until a chalk-white precipitate begins to form. The urine is now run in more slowly until one drop dissipates the last trace of color, indicating the end point in the reaction.

Calculation.—Since the 5 c.c. of the Benedict solution require exactly 10 mg. (0.01 gm.) of glucose for reduction, the calculation may be made very simple. The following formula may be used: $\frac{100}{\text{Titer}} \times 0.01 =$ glucose in per cent, or more simply, the urine used in c.c. may be divided into 1.

With this method the urine should be diluted when the sugar content exceeds 2.5 per cent. Under 0.5 per cent of sugar the figures are likewise inaccurate, and here Benedict's colorimetric method of estimating the sugar content of normal urine should be used.

Estimation of Sugar in Normal Urine

From 0.02 to 0.20 per cent of reducing sugars may be found in the urine of perfectly normal individuals, and even 0.3 per cent may be found in concentrated urines (sp. gr. 1.025 or above). The average 24 hour elimination is 0.8 gm. Benedict[12] has recently suggested an extremely simple technic of estimating the sugar content of normal urine. With the method described the

*Benedict's volumetric solution is permanent and is composed of 18.0 gm. of copper sulfate, 100 gm. of anhydrous or double the quantity of crystallized sodium carbonate, 200 gm. of sodium or potassium citrate, 125 gm. of potassium thiocyanate, and 5 c.c. of 5 per cent potassium ferrocyanide solution, made up to one liter with distilled water. In the preparation of the solution, the copper sulfate should be dissolved separately in about 100 to 150 c.c. of distilled water and then added slowly with constant stirring to a filtered solution (about 800 c.c.) of the other ingredients and finally made up to one liter.

color obtained from creatinine is completely dissipated by acetone. When a small plus error is of no importance the use of bone black may be omitted.

Method.—To 12-15 c.c. of urine (sp. gr. not over 1.030) in a large test tube or small Erlenmeyer flask add about 1 gm. of special bone black,* shake 1-2 minutes, let stand 10 minutes, and filter through a small dry filter paper.

From 1 to 3 c.c. of the filtrate (if less than 3 c.c. are used, make up with distilled water to 3 c.c.) are measured into a graduated test tube (the tube employed for the blood sugar estimation is satisfactory) and 1 c.c. of half saturated picric acid and 0.5 c.c. of 5 per cent sodium hydroxide added.

When the tube is ready to be put in the boiling water bath, 5 drops of acetone solution† are added, care being taken that the acetone does not fall on the inside wall of the test tube. The solution is now well mixed, the tube closed with a cotton plug and boiled for 15 minutes. At the same time 3 c.c. of a solution containing 1 mg. of pure glucose are treated in a similar sugar tube with 1 c.c. of half saturated picric acid, 0.5 c.c. of 5 per cent sodium hydroxide, 5 drops of the acetone solution and then heated in the boiling water bath for the same length of time as the unknown.

Both tubes are now cooled to room temperature, the standard made up to 20 c.c. and the unknown to 10, 15 or 20 c.c., according to the depth of color.

It is important that the strength of the unknown should closely approximate the strength of the standard. With a 1 mg. glucose standard, the unknown should contain not less than 0.75 mg. or more than 1.5 mg., and it is best not to have more than 1.25 mg.

Calculation.—For the calculation the following formula may be used:

$\frac{15}{R} \times \frac{D}{20} \times 0.001 \times \frac{1}{V} \times 100 =$ per cent of sugar in urine, in which R represents the reading of the unknown, D the dilution of the unknown, 0.001 the strength of the standard, V the volume of urine employed and 100 the factor to convert the figure to per cent.

For example, with a reading of 15, a dilution of 15 and 1 c.c. of urine employed, the formula would work out:

$\frac{15}{15} \times \frac{15}{20} \times 0.001 \times \frac{1}{1} \times 100 = 0.075$ per cent of sugar in urine.

Clinical Quantitative Test for Sugar in Urine

Benedict[13] has recently devised a simple clinical method of estimating small amounts of sugar in urine. It was devised for routine insurance work but may obviously be used for many clinical purposes.

Method.—Measure 1 c.c. of urine into a test tube graduated at 25 c.c. Add 3 c.c. of picric acid solution (2 grams of pure dry picric acid per liter) and 0.5 c.c. of 5 per cent NaOH. Add next 5 drops of 50 per cent acetone solution (prepared fresh each day by diluting acetone with an equal volume of water) and place the tubes promptly in a boiling water bath. In 10 to 15 minutes remove tubes from the bath, cool and dilute to 25 c.c. Compare this colored solution with standard solutions contained in test tubes of similar diameter prepared as follows: A 0.2 per cent solution of picramic acid in 0.5 per cent sodium carbonate, containing 30 c.c. of saturated picric acid per liter (previously made slightly alkaline or neutral with sodium carbonate) is prepared and diluted so that the color corresponds with that yielded by 0.1, 0.2, 0.3, 0.4, and 0.5 per cent solutions of glucose when treated exactly as described above for urine and diluted to 22 c.c. Estimate the amount of sugar. Amounts of sugar exceeding 0.2 per cent in urines of low specific gravity or 0.3 per cent in urines of high specific gravity (over 1.025) should be looked upon with suspicion.

*The special bone black may be prepared by treating 250 gm. of bone black with 1.5 liters of dilute hydrochloric acid (1 part of acid to 5 parts of water) and boiling for 30 minutes. The bone black is now filtered on a large Buchner funnel and washed with hot water until the filtrate is free from acid.

†The acetone solution is prepared by mixing 1 part of C. P. acetone with 1 part of distilled water. The solution should be freshly prepared at frequent intervals.

Clinical Quantitative Test for Protein in Urine

Folin[13] has devised a somewhat similar test for protein in urine.

Method.—Pipette 1 c.c. portions of the urine under examination and of 0.1, 0.2, 0.3, and 0.4 per cent protein solutions into a series of similar test tubes graduated at 25 c.c. Make all up to mark with 2 per cent sulfosalicylic acid solution and mix. Let stand for 10 to 15 minutes and compare. A stock protein solution of approximately one per cent strength is made by diluting sheep serum with 7 volumes of 15 per cent sodium chloride solution. This keeps well, and the other standards are made from it by dilution with 15 per cent sodium chloride solution.

Amounts of protein equal to 0.1 per cent or over should be looked upon with suspicion if confirmed by other clinical findings.

Estimation of Total Acetone Bodies

Van Slyke[14] has recently described accurate and reasonably simple methods of estimating the acetone bodies in urine (also in blood). The methods are based on a combination of Shaffer's oxidation of β-hydroxybutyric acid to acetone and Deniges' precipitation of acetone as a basic mercuric sulfate compound. Oxidation and precipitation are carried out simultaneously in the same solution, so that the technic is simplified to boiling the mixture for an hour and a half under a reflux condenser, and weighing the precipitate which forms. The acetone and acetoacetic acid may be determined either with the β-hydroxybutyric acid (described below) or separately. Neither the size of sample nor mode of procedure requires variation for different urines; the same process may be used for the smallest significant amounts of acetone bodies and likewise for the largest that are encountered. The precipitate is crystalline and excellently adapted to quick drying and accurate weighing. Preservatives other than toluene or copper sulfate should not be used.

*Method.**—The first step in the determination is the removal of glucose and other interfering substances. Place 25 c.c. of urine in a 250 c.c. measuring flask. Add 100 c.c. of water, 50 c.c. of copper sulfate solution, and mix. Then add 50 c.c. of 10 per cent calcium hydroxide, shake, and test with litmus. If not alkaline, add more calcium hydroxide. Dilute to the mark and let stand at least one-half hour for glucose to precipitate. Filter through a dry folded filter. This procedure will remove up to 8 per cent of glucose. Urine containing more should be diluted enough to bring the glucose down to 8 per cent. The copper treatment is depended upon to remove interfering substances other than glucose, and should therefore never be omitted, even when glucose is absent. The filtrate may be tested for glucose by boiling a little in a test tube. A precipitate of yellow cuprous oxide will be obtained if the removal has not been complete. A slight precipitate of white calcium salts always forms, but does not interfere with the detection of the yellow cuprous oxide.

To simultaneously determine the total acetone bodies (acetone, acetoacetic acid, and

*Solutions required for the determination of total acetone bodies according to Van Slyke are:

20 per cent Copper Sulfate.—200 gm. of $CuSO_4.5H_2O$ dissolved in water and made up to 1 liter.

10 per cent Mercuric Sulfate.—73 gm. of pure red mercuric oxide dissolved in 1 liter of H_2SO_4 of 4 N concentration.

50 Volume per cent Sulfuric Acid.—500 c.c. of sulfuric acid of 1.835 specific gravity, diluted to 1 liter with water. Concentration of H_2SO_4 must be readjusted if necessary to make it 17.0 N by titration.

10 per cent Calcium Hydroxide Suspension.—Mix 100 gm. of Merck's fine light "reagent" $Ca(OH)_2$ with 1 liter of water.

5 per cent Potassium Dichromate.—50 gm. $K_2Cr_2O_7$ dissolved in water and made up to 1 liter.

Combined Reagents for Total Acetone Body Determination.—1 liter of the above 50 per cent sulfuric acid. 3.5 liters of the mercuric sulfate, 10 liters of water.

hydroxybutyric acid) in one operation proceed as follows: Place in a 500 c.c. Erlenmeyer flask 25 c.c. of urine filtrate. Add 100 c.c. of water, 10 c.c. of 50 per cent sulfuric acid, and 35 c.c. of the 10 per cent mercuric sulfate. Or in place of adding the water and reagents separately, add 145 c.c. of the "combined reagents." Connect the flask with a reflux condenser having a straight condensing tube of 8 or 10 mm. diameter and heat to boiling. After boiling has begun, add 5 c.c. of the 5 per cent dichromate through the condenser tube. Continue boiling gently 1½ hours. The yellow precipitate which forms consists of the mercury sulfate-chromate compound ($3HgSO_4$.$5HgO$.$2(CH_3)_2CO$) of the preformed acetone, and of the acetone which has been formed by decomposition of acetoacetic acid and by oxidation of the hydroxybutyric acid. It is collected in a Gooch or "medium density" alundum crucible, washed with 200 c.c. of cold water, and dried for an hour at 110°. The crucible is allowed to cool in room air (a desiccator is unnecessary and undesirable) and weighed. Several precipitates may be collected, one above the other, without cleaning the crucible.

Calculation.—Since in the first operation the urine is diluted 1 to 10, the 25 c.c. of filtrate employed are equivalent to 2.5 c.c. of undiluted urine. One mg. of acetone yields 20.0 mg. of precipitate. For the calculation the following formula may be used:

$$\frac{\text{mg. of precipitate}}{20} \times \frac{\text{vol. of urine}}{2.5} = \text{mg. of total acetone bodies in specimen, calculated as acetone.}$$

PHENOLSULFONEPHTHALEIN RENAL EFFICIENCY TEST

The very valuable phenolsulfonephthalein functional kidney test was first proposed by Rowntree and Geraghty in 1910.[15] The test[16]: 20 to 30 minutes before administering the drug, the patient is given 200 to 400 c.c. of water to insure copious urinary secretion. Under aseptic precautions, a catheter is introduced into the bladder and the latter completely emptied. Noting the time, one c.c. of a carefully prepared solution of phenolsulfonephthalein, containing 6 mg. to the c.c. (may be obtained already prepared in the form of ampoules) is carefully administered, preferably intramuscularly in the lumbar muscles by means of an accurately graduated 1 or 2 c.c. syringe. (Where immediate urinary secretion is desired, as in ureteral catheterization, the drug may be given intravenously.) The urine is now allowed to drain into a test tube in which has been placed a drop of 25 per cent sodium hydroxide and the time of the appearance of the first pinkish tinge noted. A rough estimate of the time of appearance can be made by having the patient void urine at frequent intervals without the use of a catheter. Where the catheter has been employed and there is no urinary obstruction, the catheter is withdrawn at the time of the first appearance of the drug in the urine, and the patient is instructed to void into one receptacle at the end of one hour, and into another receptacle at the end of two hours. Practically, with patients who have no obstruction, fairly satisfactory results may be obtained simply by having the patient void at the end of one hour (or one hour and six minutes) and again at the end of two hours, or even more simply, once at the end of two hours. (If it is desired to distinguish between the efficiency of the two kidneys, the urine may be collected separately from each kidney by catheterizing the ureters.) The drug normally appears in the urine in five to ten minutes, 38 to 60 per cent being eliminated the first hour and 60 to 85 per cent during the two hours.

The percentage elimination is estimated by treating the urine of the two periods with sufficient strong sodium hydroxide to produce the maximum red

color and each diluted to 1000 c.c., if the depth of color will allow. This is then compared in a colorimeter with a standard solution containing 3 mg. of the drug to the liter. Usually a good match of colors may be obtained, although occasionally the 'phthalein assumes a pinkish tinge, making an exact match difficult.

Comparison in Bock-Benedict or Other Colorimeters of Plunger Type

Method.—To each of the two one-hour specimens of urine obtained after the administration of the drug (6 mg.) add 5 c.c. of 10 per cent sodium hydroxide to secure the maximum color from the indicator. Empty each of the specimens into liter graduated cylinders. Note volume, deducting 5 c.c. for alkali added. (One should always record the volume, so as to have a record of the copiousness of urination.) Wash out specimen bottles and employ the wash water to dilute the specimens in the cylinders. Dilute preferably to some round number figure such as 200, 500, 1000 c.c., approximating the depth of color of the standard.* Place the standard solution in the cell or cup of the colorimeter and set it at 15 mm. Now match up the individual specimens (filtration is often necessary), calculate the output of each hour and add the results.

Calculation.—For the calculation the following formula may be used:

$$\frac{15}{R} \times \frac{50\ (\%) \times \text{Dilution of specimen}}{1000} = \text{Percentage output of 'phthalein for specimen.}$$

For example, with a reading of 10 mm. and a dilution of 500 c.c. the formula would work out

$$\frac{15}{10} \times \frac{50 \times 500}{1000} = 37.5\%$$

Comparison in Test Tube Colorimeter

Method.—To each of the two one-hour specimens of urine obtained after the administration of the drug (6 mg.) add 5 c.c. of 10 per cent sodium hydroxide to secure the maximum color from the indicator. Empty each of the specimens into 250 c.c. graduated cylinders. Note volume, deducting 5 c.c. for the alkali added. Wash out specimen bottles and employ the wash water to dilute the specimens in the cylinders. Dilute preferably to 100 or 200 c.c. depending on the depth of color. Into the dry left hand tube pour or pipette 5 c.c. of the standard phenolsulfonephthalein* (3 mg. to 1000 c.c.) and filter and pipette exactly 5 c.c. of Specimen 1 into the right hand tube of the instrument. With diluting pipette now add water, a few drops at a time, inverting after each addition, until the depth of color in the two tubes is the same.

Calculation.—To calculate the percentage elimination in the hour specimen the following formula may be used: $\frac{C \times R_2}{20 \times R_1}$ = Percentage output of 'phthalein for specimen. In the formula, C represents the volume in c.c. to which the urine in the cylinder was diluted, 20 the percentage factor, R_1 the amount of fluid (5 c.c.) placed in the right-hand tube and R_2 the volume to which it was necessary to dilute this to match the standard. For example, with a 200 c.c. dilution of the urine, and 22 c.c. for R_2, the formula would work out $\frac{200 \times 22}{20 \times 5} = 44$ per cent.

The same procedure is employed to ascertain the percentage content of Specimen 2. These results are generally added together and the two-hour output recorded.

Occasionally the standard may be found too strong. In this case, the 5 c.c. in the left hand tube may be diluted with water to 10, 15, 20 or 25 c.c. and the result divided by 2, 3, 4 or 5 as the case may be.

*To prepare the standard phenolsulfonephthalein solution, break open one ampoule of the 'phthalein, pipette up 1 c.c. with an Ostwald-Folin pipette, and discharge into a two liter volumetric flask. Now add one liter of distilled water, and then sufficient strong sodium hydroxide solution to bring out the maximum color with the 'phthalein, and finally make up to the two liter mark with distilled water. Since the 1 c.c. from the ampoule contains 6 mg. of the drug, the solution now contains 3 mg. to the liter.

It is a matter of common observation that an exact color match in the phenolsulfonephthalein test is frequently impossible to obtain, probably because certain salts present in the urine act as buffers and prevent a complete change in the color of the indicator on the addition of alkali (i. e., prevent the pH from reaching 9 or more. For one who has had experience in matching the colors in the 'phthalein test, and endeavors to compare the depth of color, disregarding the quality of color, the error introduced by the "off" color is probably not great. (The author was able to match several specimens badly "off" color with an error of not more than 5 per cent.) It is quite disconcerting to some, however, to encounter colors that cannot

Fig. 24.—Author's modified Hellige colorimeter.

be satisfactorily matched. With the aid of the bicolorimeter described below, perfect color matches may be secured.

In the past the Hellige colorimeter has been quite extensively employed for the 'phthalein test. As has recently been pointed out by the author,[17] this instrument may be so modified as to provide for an extra wedge. A magnifying eye piece has also been provided, as shown in Fig. 24. It will be noted that space has been so economized as to take care of the two wedges in the box of a standard Hellige colorimeter. The two adjustment

screws are placed on either side of the instrument to permit the use of both hands in matching the colors.

As ordinarily employed, standard solutions used in the Hellige wedges are made of double strength. Under these conditions, with 100 at the top of the scale, a solution half the strength of the standard will read approximately 50 on the scale, full strength 100. (All wedges must be calibrated.) With the Hellige instrument the 'phthalein standard contains 6 mg., instead of 3 mg., to 1000 c.c. If the front wedge of the bicolorimeter is filled with the usual alkaline solution of the dye, while the second wedge is filled with an acid (yellow) solution of the same strength, it is possible to obtain a perfect match with all the shades of the indicator. When the wedges are properly calibrated, it is obviously possible to determine very accurately the content of the dye.

Since turbidity is generally due to phosphates, which can and should be removed by filtration, it need not interfere in obtaining a perfect color match. As pointed out on page 207, however, an instrument provided with a third wedge containing a colloidal suspension will permit perfect comparison of somewhat turbid solutions.

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APPENDIX

TYPES OF COLORIMETERS AND THEIR USE

It is perhaps appropriate that some special remarks be made regarding the very delicate quantitative colorimetric methods which have been developed for the chemical analysis of blood. Owing to their very great sensitiveness these methods yield a higher degree of accuracy with the small quantities of material available than is possible with the older gravimetric and volumetric methods. Indeed, in many instances they have made possible determinations otherwise quite impossible. Furthermore, these colorimetric methods are very rapid. All of the methods described in the main part of this book (Chapters II to IX) are colorimetric except those for the blood bicarbonate and chlorides. Such extensive use of colorimetric methods is a development of the past fifteen years. In 1904 Folin introduced his accurate and surprisingly simple colorimetric method for the estimation of creatinine in urine, and this gave a great impetus to the development of colorimetric methods. For this work Folin employed the Duboscq colorimeter and at the present time there are very few laboratories of biochemistry that do not boast one or more of these instruments. This instrument was first announced in 1854 by Jules Duboscq and is still made in the factory of his successors (Pellin). An improved form of the present French instrument is shown in Fig. 25.

Owing largely to the increase in the number of colorimetric methods and their extensive utilization for both scientific and clinical purposes, the demand for colorimeters has greatly multiplied. Until 1915 the instruments in use in this country were all of foreign manufacture (Pellin, Krüse, Hellige), but since that time a number of American concerns (Klett, Eimer & Amend, Leitz—N. Y., Bausch & Lomb, Spencer) have undertaken the manufacture of such instruments.

Colorimeters

The various colorimeters may be simply designated as of the (1) plunger, (2) wedge or (3) dilution type. With the plunger type the intensity of the color of either the standard or unknown is varied by changing the depth of solution through which the light passes with the aid of a plunger. The Duboscq in its various forms, the Klett and Bock-Benedict are of this type, although with the last named instrument a plunger is used for the unknown only.

In the case of instruments constructed on the Duboscq pattern, light from some even source of illumination is passed through the two sides of the instrument. The solutions to be compared are placed in cups in these two light paths. Some of the light in passing through the liquids is ab-

sorbed, the amount of absorption depending on the depth of the solution. The two beams of light are now brought to a common axis by means of rhombohedral prisms. Light from one cup illuminates one-half of a circular field, and light from the other cup illuminates the other half. The observing microscope, by which the observer sees both fields with one eye, is focused on the line of separation of the two fields. It is now possible to alter the depths of the columns of liquid until the two halves of the field are identical in intensity. When this condition holds, the concentrations of the two solutions are inversely proportional to their depths, which may then be read on the vernier scales of the instruments.

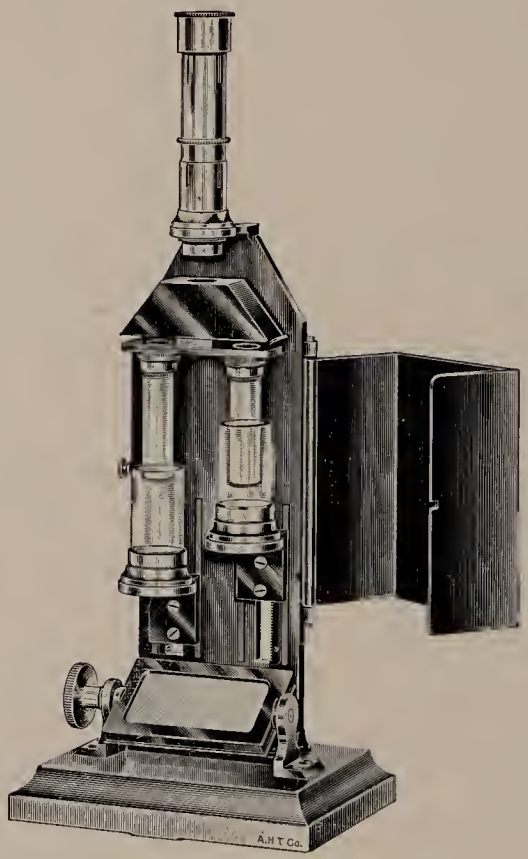

Fig. 25.—Duboscq colorimeter (Pellin).

With a colorimeter of the wedge type such as the Hellige, the standard is placed in a wedge and the unknown in a small cup with sides parallel to the wedge. The wedge is moved up and down until the intensity of color is the same as the unknown. The two solutions are brought together for comparison with the aid of Helmholtz prisms.

With the dilution type of instrument such as the Sahli hemoglobinometer and the inexpensive test tube instrument described by the writer, the unknown is diluted with water or some diluting fluid until it has identically the same color as the standard.

Colorimeters of the Plunger Type

Instruments of the plunger type have been most extensively employed where accuracy and rapidity are required. Of the individual instruments

on the market, the Duboscq, as pointed out above, is the oldest and best known. The instruments on the Duboscq principle are made in several different sizes, i. e., to accommodate cups 20, 40, 50, 65 and 100 mm. in height. The colorimetric methods described for various biochemical analyses seldom necessitate working with very lightly colored solutions. Consequently the large 100 mm. instruments are not necessary, and, as a matter of fact, their size makes them quite cumbersome to operate. Many biochemical laboratories appear to have purchased these large colorimeters under the impression that they were securing a superior instrument, but they possess no advantage aside from their ability to accommodate a greater depth of solution. In special instances where dilute solutions need to be matched, taller cups may be employed in the 50 mm. instruments. The author has used such cups with the Klett instrument.

It is difficult to compare the various 50 mm. instruments on the market at the present time, and an attempt will be made simply to point out the special features of the different colorimeters. Although the cups of the Klett instrument are slightly taller than those of the various 50 mm. Duboscq colorimeters, the instrument is essentially the same size and belongs in the same category. The same is true of the B. & L. Biological, and the Bock-Benedict. Although the cups in these instruments are slightly smaller, they are large enough to be adequate for practically all biochemical determinations. Although the more expensive instruments in this group possess added features and refinements and are larger, it is doubtful if they will yield any more accurate results than some of the smaller and cheaper instruments.

The original French instrument (Pellin) is very substantially constructed (see Fig. 25) and the glass parts are of excellent quality. This latter fact is very important, since good prisms are probably the most essential part of the instrument, otherwise it would not be possible to make the two halves of the field appear the same. Disadvantages of this instrument are the high cost and the fact that the cups and plungers are mounted in balsam and require frequent remounting. (The author has found it advantageous to remount the plungers and cups, when they become loose, in plaster of Paris or sealing wax. In using plaster of Paris, sufficient water should be employed so that the mixture will just flow. If it is too stiff, it cannot be properly manipulated.) The instrument manufactured by Eimer & Amend is practically an exact copy of the French Duboscq, except that cups and plungers are mounted in plaster of Paris and the vernier scale is adjustable. The Krüse model of the Duboscq, available before the war, contained some advantages over the French instrument, notably in the mounting of the cups and plungers, and was more reasonable in price, although in general it was hardly the equal of the Duboscq.

The American-made Klett (see Fig. 26), originally designed by P. A. Kober, has a number of mechanical advantages over the French Duboscq and is less expensive. It has a somewhat better arrangement for adjusting the stages than the old style rack and pinion and the reflecting mirrors are divided, to permit a more even adjustment of the light of the two fields.

The cups and plungers are of black glass with fused-on clear glass bottoms, thus removing all light except that passing from the reflecting mirrors to the prisms. Since the cups are fused and not mounted in balsam, chloroform may be used with impunity. It should be noted, however, that the cups are much more fragile than in the Duboscq. (Metal encased cups may be obtained with the instrument if desired). The prisms are somewhat smaller than those used in the French instrument, and it seems doubtful if the fields are quite as evenly matched. Kober and Klett[1] have recently introduced a device containing a reflecting mirror (see Fig. 26) whereby readings may be made without materially changing the position of the eye. They have also designed a similar device for attachment to the standard

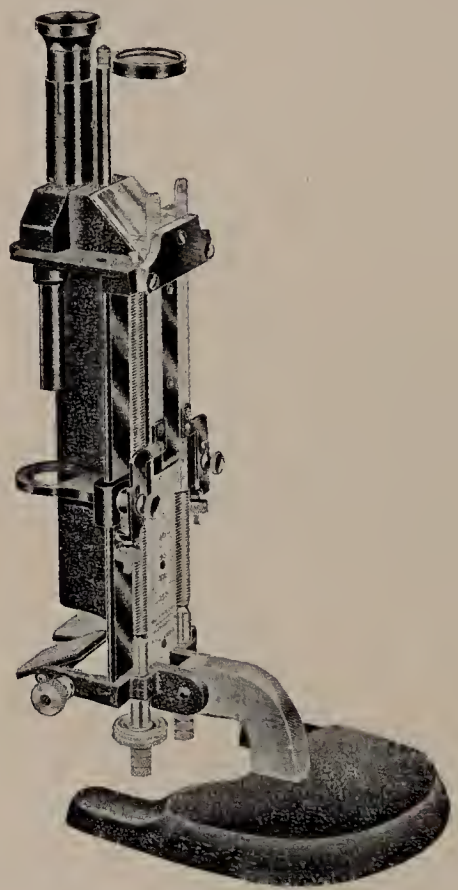

Fig. 26.—Klett colorimeter with top reader.

Duboscq instrument. This unquestionably saves some time in making readings and is very convenient.

Bausch and Lomb have rather recently entered the colorimeter field, and manufacture a 50 mm. instrument similar to the original Duboscq but designed to meet the most exacting requirements. Into its construction are built such refinements as optically inactive tube bottoms, plungers of optical glass matched for color, adjustments of microscopic precision and a dust-proof housing for the prism system. The verniers are provided with a reading mirror set at forty-five degrees, enabling the observer to make the scale reading with only a slight shift of the eye to the side. The verniers are adjustable. Although the comparative cost of this colorimeter is

rather high, it can hardly be said to be out of proportion to its quality.

Bausch and Lomb also make a smaller instrument, illustrated in Fig 27, which has been designated, the Biological Colorimeter. It is appropriately named, as the instrument is well suited to biochemical work. It possesses practically all the advantages of the larger instrument although it sells for less than half the price. The compound prism of this instrument is of the overlapping type, in which the dividing line is a silver edge and practically invisible. The plungers are made of optical glass of high light transmission, free from color and are six-sided in form so that light is deflected and enters the prism only from the bottom. The cups, in metal casings, have plane parallel bottoms and are separable for cleaning. This design was suggested by Dr. Folin. The right-hand cup, containing the "unknown," is moved by rack and pinion, while the left-hand cup, contain-

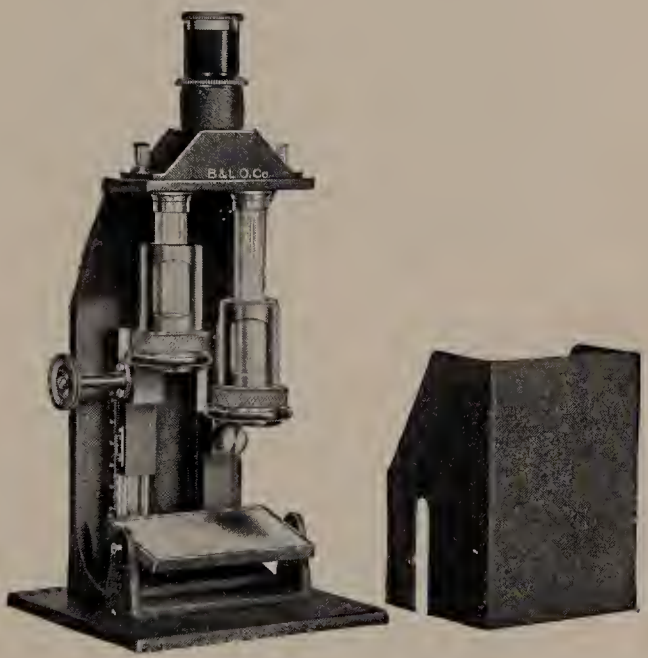

Fig. 27.—Biological colorimeter (B. & L).

ing the "standard," is held at any desired position by means of a thumb screw. Although the omission of the rack and pinion on the left-hand cup was made as an economy, and may be supplied if desired, it has the important advantage that when the standard is once set in place, it cannot be jarred out of position. The field in this instrument is large and, owing chiefly to the excellent prisms, is very evenly matched, making accurate readings comparatively easy. Since the stand of this instrument is very low, readings can conveniently be made from a sitting position. As pointed out on page 167, the insertion of a colored glass plate on the top of the left hand prism, following Newcomer's suggestion, converts the instrument into an accurate hemoglobinometer.

Very recently the Spencer Lens Co. has put out a colorimeter on the Duboscq pattern (illustrated in Fig. 28), which accommodates 50 mm. cups and still is comparatively small in size and quite reasonable in price.

The cups are mounted in a metal base with plaster of Paris, and with a slight turn lock in place so that they cannot fall out if the instrument is tipped. The positions of both cups are adjustable by independent rack and

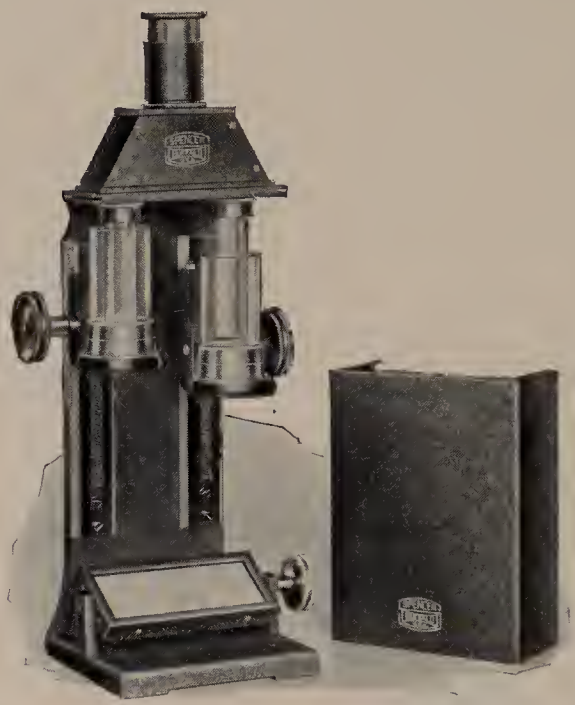

Fig. 28.—Spencer Duboscq colorimeter.

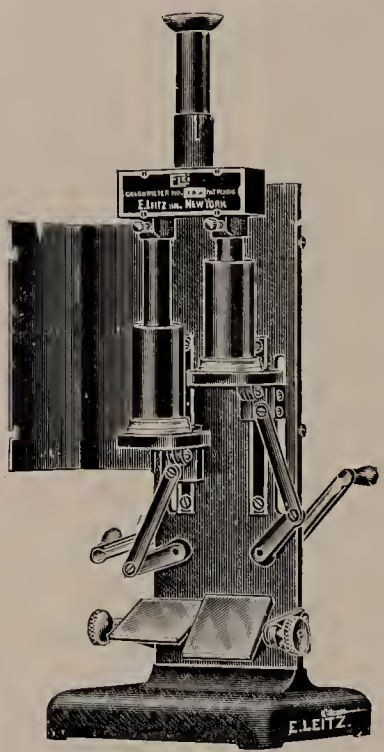

Fig. 29—Elei colorimeter.

pinion movements, the rack and pinion being very accurately constructed. The plungers of solid glass are round. It possibly would have been an advantage to have made them hexagonal to deflect light coming from the

side. The stand is sufficiently low to permit use of the instrument in a sitting position.

For several years E. Leitz, N. Y., has manufactured an instrument on the Duboscq pattern which they have called the Elei colorimeter (see Fig. 29). This is a comparatively small and compact instrument, accommodating 50 mm. cups. The cylinders of the plungers and cups are of opaque glass with clear glass bottoms. The bottoms of the cups are fused on and then mounted in a metal base with plaster of Paris. The usual prism system is employed. For the customary rack and pinion movement, a quick-acting but extremely sensitive lever device has been substituted. Owing possibly to this economy the price of the Elei colorimeter is below that of the various instruments mentioned above.

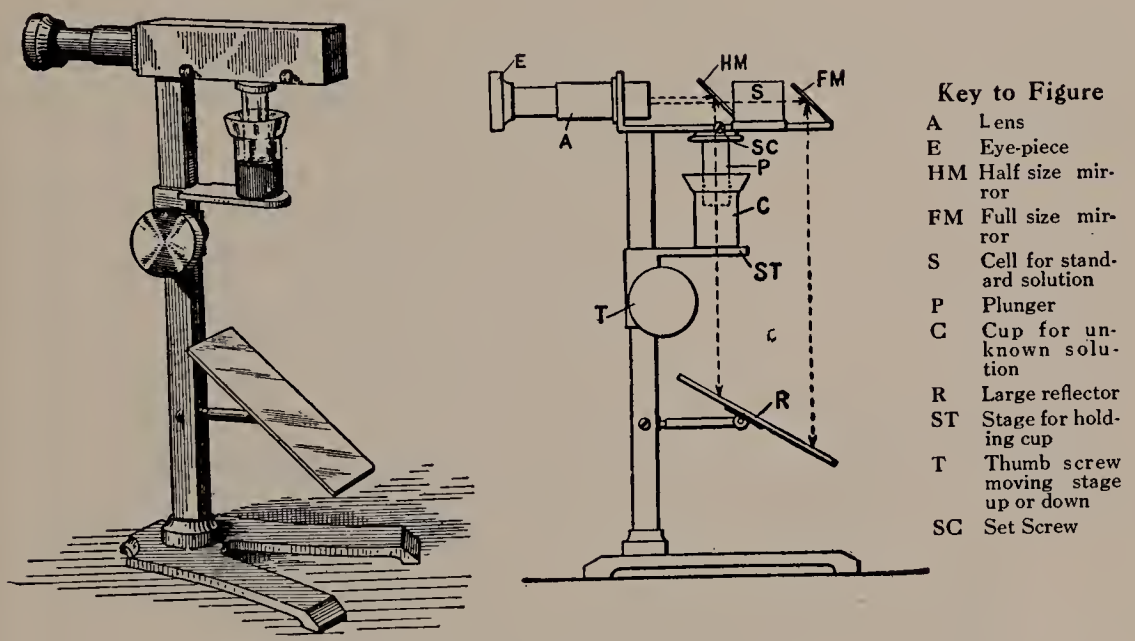

Fig. 30.—Bock-Benedict colorimeter.

A simple and relatively inexpensive colorimeter has recently been introduced by Bock and Benedict,[2] and is now made by Klett Mfg. Co. (see Fig. 30). In this instrument small reflecting mirrors have been substituted for the expensive prisms. The standard is put in a cell of known dimensions through which the rays of light pass longitudinally, thus removing the necessity for more than one moving part. Colorimetric estimations may be made very rapidly with this instrument, and it is possible, when the mirrors are properly matched to make very accurate color comparisons. To some the position of the eye-piece may not appear convenient, but we have found that when the instrument is placed on a shelf level with the eye facing the light (north), it can be very easily manipulated. The cup of the instrument is small and when selected to closely fit the plunger, satisfactory comparisons may be made with 2 c.c. of fluid, as small an amount as can be handled in the so-called microcolorimeters. Although the colorimeter gives very satisfactory service when carefully handled, it will not

stand hard and careless use. Fortunately the mirrors, cup and cell can be easily and cheaply replaced. However, when carelessly used, it is difficult to keep the instrument in proper adjustment.

The Duboscq biological colorimeter (Pellin) with 20 mm. cups seems to fall just short of being large enough to be useful in biochemical work.

Hellige Wedge Colorimeter

The Autenrieth-Königsberger colorimeter of Hellige has the advantage that permanent standards may be kept in individual wedges, but the disadvantages that few of these standards are really permanent and that the wedges need to be empirically standardized. When a wedge is carefully calibrated for a given purpose by the individual using the instrument, very satisfactory results may be obtained. However, just the individuals to whom this instrument especially appeals are the ones who will not insure the accurate calibration of the standard wedges. The instrument is very well suited for certain special micro-determinations, but is not as satisfactory for general biochemical use as instruments made on the Duboscq pattern.

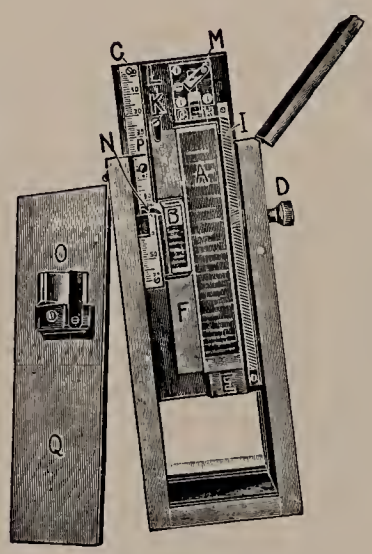

Fig. 31.—Hellige colorimeter.

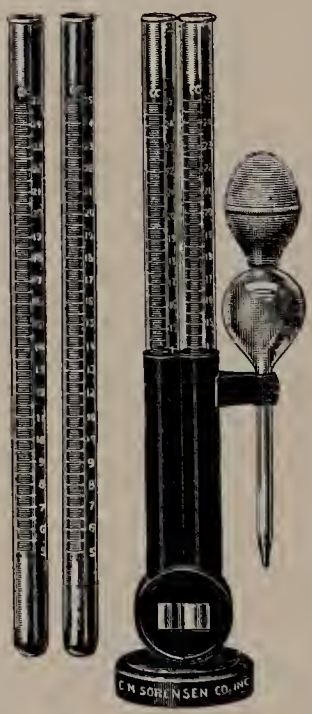

Fig. 32.—Test tube colorimeter

Dilution Type of Colorimeter

With the dilution type of instrument such as the Sahli hemoglobinometer and the inexpensive test tube instrument (see Fig. 32) described by the writer,[3] the unknown is diluted with water or some diluting fluid until it has identically the same color as the standard. Theoretically, the principle of diluting the unknown to the same intensity of color as the standard is excellent, but practically, it requires considerable care in execution and fairly large volumes of fluid in order to secure accuracy, since

near the end point the diluting fluid must be added a drop or a few drops at a time. Where the volume of fluid is large enough that one or two drops of fluid more or less do not impair the accuracy of the test, as in the test tube instrument mentioned above, the method is perfectly satisfactory, especially for an occasional estimation, but in such very small instruments as the Sahli and Kuttner a large error is necessarily introduced.

Bicolorimeter

There are many purposes for which a colorimeter employing a single color standard is inadequate. One of the best illustrations is the color

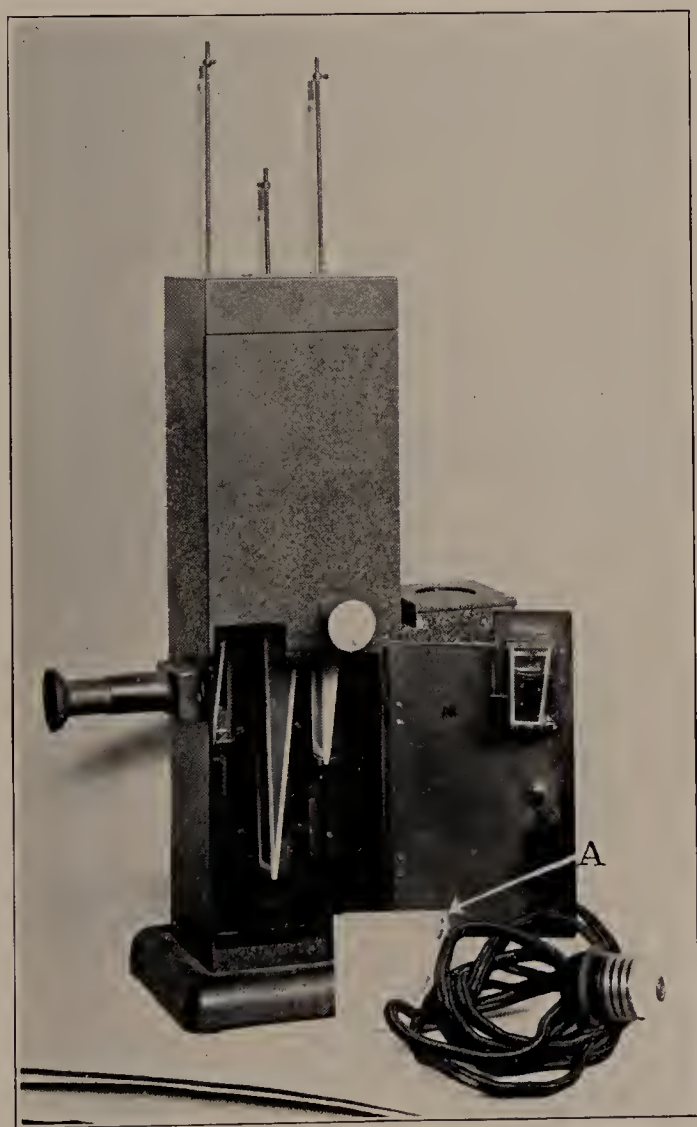

Fig. 33.—Myers-Leitz Bicolorimeter (A, milk-glass plate which may be substituted for lamp box).

imetric determination of the hydrogen ion concentration. As ordinarily carried out this determination is cumbersome and rather crude, since it has been necessary to employ a series of tubes with graduated pH values as standards, owing to the fact that here one is dealing with combinations of two colors instead of a single color.

It has been pointed out by the author[4] that with the use of two (Hellige) wedges in a special colorimeter, it is possible to match all shades of color in a given indicator from the acid to the alkaline side, when one wedge is filled with an acid solution of the dye and the other with the alkaline solution, thus reducing the number of standards employed with a single indicator to two.

A new wedge colorimeter, designed especially for bicolorimetric work has been described by the author[4] (see Fig. 33). This instrument comprises a brass box with heavy metal base, and contains a rack and pinion arrangement for three wedges, the movement of the wedges being entirely within the closed box. Readings are taken from 100 mm. (adjustable) scales which emerge from the top of the instrument as the wedges are raised. The same type of rhombohedral prisms employed in the colorimeters constructed on the Duboscq pattern have been employed to bring the light passing through the wedges and the cup containing the unknown into the same observation field for comparison. A milk-glass plate (shown by A in Fig. 33) in back allows for the entrance of light. A small lamp box may be substituted for this. Only reflected light is used, which passes through a thick daylight glass. The light obtained compares favorably with daylight in quality although somewhat greater in intensity. A door at the side of the instrument gives access to the wedges and to the cup for the unknown which is mounted on it. Two wedges provide for bicolorimetric work as in the pH determination. However, to obtain a perfect match with unknown solutions which are slightly turbid or colored a third wedge may be used. To secure an even field it is sometimes necessary to counterbalance the greater thickness of glass on the wedge side of the field. (The wedges used by the author were constructed by E. Leitz, N. Y., of somewhat thinner optical glass than ordinarily employed in the Hellige wedges.) Provision has been made for this by attaching to the rack carrying the cup for the unknown, a clip which will hold one or more glass plates.

With the aid of this instrument very accurate estimations of the hydrogen ion concentration of blood may be made. It is also well suited for pH determinations in urine, gastric contents and bacteriological culture media. Although the instrument was not designed for ordinary colorimetric work, very accurate colorimetric comparisons may be made when only the first wedge is used. Obviously the same criticisms apply to its use as a colorimeter as in the case of the Hellige instrument.

Nephelometers

Nephelometric attachments may be obtained for the Duboscq (Pellin) and are constructed for the Klett, B. & L. 50 mm., Duboscq and Biological and the Elei colorimeters. In some cases the lamp employed for the nephelometric work may also be utilized as the artificial illumination for colorimetric work.

For the convenience of those who may be in need of a colorimeter, the latest retail prices of various instruments together with the names of the

manufacturers are given in the table below. The instruments may be secured from practically any dealer in laboratory supplies.

MANUFACTURERS AND PRICES OF VARIOUS COLORIMETERS

INSTRUMENT	MAKER	RETAIL PRICE
Duboscq, 100 mm.	Bausch and Lomb	$165.00
" " "	Pellin, Paris	163.75*
" " "	E. Leitz, N. Y.	140.00
Klett, 65 mm., with top reader	Klett	90.00
" " " without top reader	Klett	75.00
Duboscq, 50 mm.	Bausch and Lomb	125.00
" " "	Pellin, Paris	118.75*
" " "	Eimer and Amend	118.75
" " "	Spencer	70.00
Elei, " "	E. Leitz, N. Y.	50.00
Biological, 40 mm.	Bausch and Lomb	60.00
Bock-Benedict	Klett	40.00
Duboscq-Biological, 20 mm.	Pellin, Paris	75.00*
Hellige	Hellige & Co.	38.50†
Myers Test-Tube	Klett	12.00
Myers Bicolorimeter	E. Leitz, N. Y.	

*Prices quoted by Arthur H. Thomas Co., Phila.
†Price quoted by E. Leitz, N. Y.

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APPENDIX B

STANDARD SOLUTIONS AND REAGENTS*

Acetic Acid-Acetate Solution (for Folin's amino acid N determination).—Dilute 100 c.c. of 50 per cent acetic acid with an equal volume of 5 per cent sodium acetate solution. The presence of the sodium acetate in this solution serves two purposes: (1) to increase unmistakably the color of the quinone-amino acid derivative, and (2) to retard very much the onset of turbidity due to the liberation of sulfur from the added sodium thiosulfate. Both of these results are due to the weakened acidity of the acetic acid.

Acetic Anhydride (for cholesterol).—Highest purity of reagent required.

Acetone Solution (for sugar in normal urine).—The acetone solution is prepared by mixing 1 part of c.p. acetone with 1 part of distilled water. The solution should be freshly prepared at frequent intervals.

Amino Acid Solution, Standard (Folin).—The standard solution of amino acid used in blood analysis should contain 0.07 mg. of nitrogen per c.c. It is well to make up a stock solution containing 0.1 mg. per c.c. The solution is made with 0.1 N hydrochloric acid, and 0.2 per cent of sodium benzoate, in which amino acids seem to keep indefinitely. Glycine (glycocoll) is the amino acid which can probably be used to best advantage. Since glycine contains 18.7 per cent nitrogen, 268 mg. would be required to make 500 c.c. of a solution containing 0.1 mg. N per c.c. From the stock solution containing 0.1 mg. of nitrogen per c.c. the blood standard is made by diluting 70 c.c. with 0.1 N hydrochloric acid to a volume of 100 c.c. It is obvious that the amino acid employed as a standard should be of known purity.

Ammonia Water, 1 per cent, Carbonate Free (for CO_2 determination).—Ordinary ammonia solution can be made carbonate-free by adding a small amount of saturated barium hydrate solution. The barium carbonate is filtered off, and the excess of barium remaining is precipitated with a little ammonium sulfate. The ammonia can then be diluted to the desired strength.

Ammonium Thiocyanate Standard (for chlorides).—See chloride standards.

Amyl Alcohol.—Highest purity, used as foam inhibitor in estimation of urea in blood and urea and ammonia in urine.

Benedict's Qualitative Solution (for sugar).—The solution is composed of 17.3 grams of copper sulfate, 173.0 gm. of sodium citrate and 100 grams of

*In a moderately well equipped laboratory there should be little difficulty in the preparation of the various standard solutions and reagents listed below, and one derives considerable satisfaction in using solutions which have been personally prepared and which are known to be correct.

There are quite a good many individuals, however, who do not have the time or possess all the necessary equipment, such as an accurate quantitative balance, etc., to make up some of these standard solutions. At the suggestion of the author and as a service to such clinical laboratory workers, the standard solutions and reagents listed below are now prepared by the Sorensen Scientific Corporation, 444 Jackson Ave., Long Island City, N. Y.

anhydrous sodium carbonate (double the weight of the crystalline salt may be employed) made up to one liter with distilled water. In the preparation of the solution, the copper sulfate should be dissolved separately in about 100-150 c.c. of distilled water and then added slowly with constant stirring to a filtered solution (about 800 c.c.) of the other ingredients and finally made up to one liter.

Benedict's Volumetric Solution (for sugar in urine).—This is likewise permanent and is composed of 18.0 gm. of copper sulfate, 100 gm. of anhydrous or double the quantity of crystallized sodium carbonate, 200 gm. of sodium citrate, 125 gm. of potassium thiocyanate, and 5 c.c. of 5 per cent potassium ferrocyanide solution, made up to one liter with distilled water. In preparation, the ingredients are dissolved in the same manner as the qualitative reagent, i.e., the copper separately.

Bone Black (for sugar in normal urine).—The special bone black is prepared by treating 250 gm. of bone black with 1.5 liters of dilute hydrochloric acid (1 part of acid to 5 parts of water) and boiling for 30 minutes. The bone black is now filtered on a large Buchner funnel and washed with hot water until the filtrate is free from acid.

Buffer Mixtures (for urea determination).—Folin suggests two buffer mixtures for urease decomposition. To preserve a substantially neutral reaction during the decomposition of urea by means of urease, mixtures of mono- and disodium phosphates in the proportion of 1 molecule of the former to 2 of the latter, and in molar concentration, are usually employed.

(a) Dissolve 69 gm. of monosodium phosphate and 179 gm. of crystallized disodium phosphate in 800 c.c. of warm distilled water. Cool and dilute to a volume of 1 liter. Preserve with 1 to 2 c.c. toluene.

Folin questions whether the maintenance of neutrality is adequate to explain fully the accelerating action of phosphates on the urea decomposition, because pyro- and metaphosphates seem to be more effective than orthophosphates. An excellent buffer mixture may be prepared as follows:

(b) Dissolve 14 gm. of sodium pyrophosphate ($Na_4P_2O_7$, 10 H_2O) in enough half normal phosphoric acid to make a volume of 100 c.c. The half normal phosphoric acid is made by diluting 200 c.c. of 85 per cent phosphoric acid to 1 liter and titrating 5 c.c. with tenth normal alkali, and phenolphthalein as indicator, to a faint pink color. On the basis of the titration, dilute the acid to a substantially correct half normal solution. The pyrophosphate-phosphoric acid mixture gives a faint color with rosolic acid. Five c.c. when titrated with tenth normal alkali and phenolphthalein should give a faint but distinct color with about 18 c.c. of the alkali.

Caprylic Alcohol (for CO_2 and O_2 determination).—Used as foam inhibitor; may be used for urea estimation also, when pure.

Chloride Standards.—(1) Blood.

(a) The standard silver nitrate-acidified ferric alum indicator solution may be prepared by dissolving 2.906 gm. silver nitrate in distilled water and making up to 1000 c.c. and then mixing with 1000 c.c. of acidified indicator containing 100 gm. of crystalline ferric ammonium sulfate and 100 c.c. of 25 per cent nitric

acid. Both the silver nitrate and the acidified indicator solution are one-tenth (combined one-twentieth) the strength of similar solutions employed for the urine, and can therefore be prepared by simply diluting the urine solutions. Each c.c. of the combined solution is equivalent to 0.0005 gm. of sodium chloride.

(b) The ammonium thiocyanate solution is standardized against the silver nitrate and made of equivalent strength. It contains approximately 0.65 gm. of the thiocyanate to 1000 c.c. It is one-twentieth of the strength employed for urine.

(2) Urine.

(a) The silver nitrate standard is prepared by dissolving 29.06 gm. of pure silver nitrate in and making up to 1000 c.c. with distilled water. Each c.c. of such a solution is equivalent to 0.01 gm. of sodium chloride.

(b) The acidified indicator is prepared by dissolving 100 gm. of crystalline ferric ammonium sulfate in 100 c.c. of 25 per cent nitric acid.

(c) The standard ammonium thiocyanate is prepared by dissolving about 13 gm. in 800 c.c. of distilled water and then determining according to the method described for the titration (see p. 140) how much of the solution is required to completely precipitate 10 c.c. of the silver solution. From this one may calculate how much water must be added to the remainder of the thiocyanate solution to make it exactly equivalent to the standard silver solution.

Chloroform (for cholesterol).—This needs to be pure and anhydrous; can best be redistilled.

Cholesterol.—Pure crystals are needed to standardize the naphthol green B in the cholesterol estimation.

Colloidal Iron (for urea in saliva).—Merck's 5 per cent Fe_2O_3 is used.

Copper Sulfate (as catalyzer in nitrogen determinations).—10 per cent solution.

Copper Reagent, Folin-Wu's Alkaline (for blood sugar determination).—Dissolve 40 gm. of anhydrous sodium carbonate in about 400 c.c. of water, and transfer to a liter flask. Add 7.5 gm. of tartaric acid, and when the latter has dissolved, add 4.5 gm. of crystallized copper sulfate; mix and make up to a volume of 1 liter. If the carbonate is impure a sediment may form after a time. If this happens, decant the clear solution into another bottle.

Creatinine Standard Solution (for creatinine in blood).—Creatinine may now readily be prepared perfectly pure by the admirable method of Benedict (Jour. Biol. Chem., 1914, xxiii, 183). A standard solution of this creatinine, 1 mg. to 1 c.c. is kept in 0.1 N hydrochloric acid. This may be prepared by dissolving 100 mg. of creatinine in water in a 100 c.c. volumetric flask, adding 1 c.c. of concentrated hydrochloric acid and making up to volume.

From this stock solution one can readily prepare standards containing 0.3, 0.5 and 1.0 mg. creatinine to 100 c.c. of saturated picric acid solution. These solutions should not be exposed to direct sunlight and should be renewed once in two months. They can be prepared in 200 c.c. quantities by pipetting 0.6, 1.0 and 2.0 c.c. respectively of the stock solution with a Mohr pipette into 200 c.c.

volumetric flasks and making up to the mark with fresh saturated (1.2 per cent) picric acid solution.

Satisfactory standard solutions for blood work may likewise be prepared directly from urine, by diluting with picric acid after ascertaining the content of creatinine by the original Folin method (for technic see p. 190).

Creatinine Standard, Folin's.—A standard creatinine solution, suitable both for creatinine and for creatine determinations in blood, can be made as follows: Transfer to a liter flask 6 c.c. of a standard creatinine solution containing 1 mg. creatinine per c.c. (See p. 212.) Add 1 c.c. of pure concentrated hydrochloric acid, dilute to the mark with water, and mix. Transfer to a bottle and add four or five drops of toluene or xylene. Five c.c. of this solution contain 0.03 mg. of creatinine, and this amount plus 15 c.c. of water represents the standard needed for the vast majority of human bloods, for it covers the range of 1 to 2 mg. per 100 c.c. In the case of unusual blood representing retention of creatinine, take 10 c.c. of the standard plus 10 c.c. of water, which covers the range of 2 to 4 mg. of creatinine per 100 c.c. of blood; or 15 c.c. of the standard plus 5 c.c. of water by which 4 to 6 mg. can be estimated. By taking the full 20 c.c. volume from the standard solution at least 8 mg. can be estimated; but when working with such blood, it is well to consider whether it may not be more advantageous to substitute 5 c.c. of blood filtrate plus 5 c.c. of water for the usual 10 c.c. of filtrate.

Digestion Mixture, Folin's (for total N).—Mix 300 c.c. of phosphoric acid syrup (85 per cent) with 100 c.c. of concentrated sulfuric acid. Transfer to a tall cylinder, cover well to exclude the absorption of ammonia, and set aside for sedimentation of calcium sulfate. This sedimentation is very slow, but in the course of a week or so the top part is clear and 50 to 100 c.c. can be removed by means of a pipette. (It is not absolutely necessary that the calcium should be thus removed, but is probably a little safer to have it done.) To 100 c.c. of the clear acid add 10 c.c. of 6 per cent copper sulfate solution and 100 c.c. of water. One c.c. of this solution is employed for the digestion.

Glucose (for glucose tolerance test).—Commercial glucose syrup answers very well for this purpose. This is ordinarily about 90 per cent. To determine the exact strength add about one-third water, and then with an Ostwald-Folin pipette, measure 1 c.c. and dilute to 100. Determine the strength of this solution just as described for blood sugar (see p. 92). Now dilute to 50 per cent strength. For the test administer 1.75 gm. per kg. of body wt.

Glucose Standard (for sugar in normal urine).—This standard may be prepared by dissolving 167 mg. of pure glucose in 500 c.c. of distilled water. About 5 c.c. of toluene should be added and the solution preferably kept in the ice box.

Glucose Standard (for blood sugar).—This is prepared by dissolving 0.100 gm. of pure glucose in 500 c.c. of saturated picric acid solution, i.e., making a 0.02 per cent solution. Three c.c. of this solution contain 0.6 mg. glucose.

Glucose, Standard (for Folin-Wu blood sugar determination).—Dissolve 2.5 gm. of benzoic acid in 1 liter of boiling water and cool. Transfer to a bottle; the solution will keep indefinitely.

Dissolve 1 gm. of pure glucose in about 50 c.c. of the benzoic acid solution. Transfer to a 100 c.c. volumetric flask, rinse, and fill to the mark with the benzoic acid solution. This is the 1 per cent standard stock solution and appears to keep indefinitely.

Transfer 1 c.c. of the stock solution, by means of an Ostwald-Folin pipette to a 100 c.c. volumetric flask; fill to the mark with saturated benzoic acid and mix. The diluted solution so obtained, which contains 0.1 mg. of glucose per c.c., is a suitable standard for most blood sugar determinations. Another standard, twice as strong, is occasionally needed. This is made by diluting 2 c.c. of the stock solution to 100 c.c. with the benzoic acid solution. Use 2 c.c. for each determination.

Hydrochloric Acid (for urea).—10 per cent solution.

Hydrochloric Acid (for creatine in urine).—Normal solution.

Hydrogen Peroxide (for nitrogen).—Concentrated, 3 per cent solution.

Hydroquinone-Bisulfite Reagent, Benedict's (for phosphorus in blood).—This reagent may be prepared by dissolving 30 gm. of sodium bisulfite and 1 gm. hydroquinone in water and making up to 200 c.c.

Lead, Powdered Metallic.—For creatine in urine.

Lithium Oxalate (used as anticoagulant by Folin).—Since lithium oxalate is apparently unavailable in the market, Folin suggests the following method of preparation: To 50 gm. of lithium carbonate in a liter beaker add 85 gm. crystallized oxalic acid. Pour on the mixture about 1 liter of hot water (70°C.). Stir cautiously, to avoid loss of liquid by foaming. Evaporate the resulting solution to dryness, and powder.

To prepare the oxalate cloth, cut 80 gm. bird's-eye cotton cloth, free from starch, into strips 10 cm. by 40 to 50 cm. Transfer 10 gm. of lithium carbonate and 17 gm. of oxalic acid to a liter beaker. Add 240 c.c. of water (70°C.) and shake. Transfer the solution to a plate. Draw the cotton strips through it, and hang up to dry. The cloth when dry will contain about 20 per cent of lithium oxalate, and a very small piece (50 mg.) is adequate for 15 or even 20 c.c. of blood. Since the lithium oxalate solution is practically saturated, the cloth may be given an additional charge of oxalate by repeating the above process, if desired.

Metallic Mercury.—Used in Van Slyke gas analysis apparatus.

Mineral Oil.—For CO_2 and pH estimation in plasma.

Molybdic Acid Reagent, Benedict's (for phosphorus in blood).—Dissolve 20 gm. of molybdic acid (Eimer and Amend 99.9 per cent pure) in 25 c.c. of 20 per cent sodium hydroxide with warming. Dilute to 200 c.c. and filter if necessary. Transfer to a liter flask and add with constant agitation under cold running water an equal volume (about 200 c.c.) of concentrated sulfuric acid.

The reagent possesses a fairly deep blue color, but on dilution with the unknown or standard previous to heating the color disappears.

Naphthol Green B (for cholesterol).—A 0.005 per cent solution of naphthol green B may be used as the standard in the estimation of cholesterol. It is

standardized against the color developed on 5 c.c. of chloroform containing 0.4 mg. of cholesterol.

β-Naphthoquinone-Sulfonic Acid, Fresh 0.5 Per Cent Solution of the Sodium Salt of (for amino acid N determination).—Owing to the difficulties in securing this quinone, Folin has described a reasonably simple synthesis, by which enough of this compound for several thousand amino acid determinations can be made in the course of two mornings. In solution β-naphthoquinone-sulfonic acid is gradually decomposed and the solution becomes visibly darker in the course of a few hours, particularly if not kept in the dark. For this reason only freshly prepared solutions should be used. The samples need not be weighed any more accurately than can be done on a small torsion balance, because a variation either way of 5 per cent makes no difference.

Transfer 100 mg. of the quinone to a small flask, add 20 c.c. of water and shake. Complete solution is obtained almost at once. For miscellaneous amino-acid determinations when 0.1 mg. of nitrogen is the standard, 3 c.c. of the reagent are taken, for 5 c.c. of blood filtrate only 1 c.c.

Nessler's Solution (for ammonia N in total N, urea, etc.).—Bock and Benedict formula: Place 100 gm. mercuric iodide and 70 gm. potassium iodide in a liter volumetric flask and add about 400 c.c. of water. Rotate until solution is complete. Now dissolve 100 gm. sodium hydroxide in about 500 c.c. water, cool thoroughly, and add with constant shaking to the mixture in the flask; then make up with water to the liter mark. This usually becomes perfectly clear. When the small amount of dark brownish red precipitate, which forms, settles out, the supernatant fluid is ready to be poured off and used.

When pure mercuric iodide cannot be obtained Folin and Wu have pointed out that metallic mercury and iodide may be substituted. To 75 gm. of potassium iodide and 55 gm. iodine in a 500 c.c. flask add 50 c.c. water and an excess of metallic mercury, 75 gm. Shake the flask for 7 to 15 minutes or until the dissolved iodine has nearly disappeared. When the red iodine solution has begun to become visibly pale, though still red, cool in running water and continue the shaking until the reddish color of the iodine has been replaced by the greenish color of the double iodide. Now separate the solution from the surplus mercury by decantation and washing with about 400 c.c. of distilled water. Add 500 c.c. of water containing 100 gm. of sodium hydroxide as in the first formula and make up to 1 liter.

Nessler's Reagent (Folin's modified).—This reagent is essentially a solution of the double iodide of mercury and potassium (HgI_2, 2KI) containing sodium or potassium hydroxide. Folin's method of preparing the double iodide is as follows: Transfer 150 gm. of potassium iodide and 110 gm. of iodine to a 500 c.c. Florence flask; add 100 c.c. of water and an excess of metallic mercury, 140 to 150 gm. Shake the flask continuously and vigorously for 7 to 15 minutes, or until the dissolved iodine has nearly all disappeared. The solution becomes hot. When the red iodine solution has begun to become visibly pale, though still red, cool in running water, and continue the shaking until the reddish color of the iodine has been replaced

by the greenish color of the double iodide. This whole operation usually does not take more than 15 minutes. Now separate the solution from the surplus mercury by decantation and washing with liberal quantities of distilled water. Dilute the solution and washings to a volume of two liters. If the cooling was begun in time, the resulting reagent is clear enough for immediate dilution with 10 per cent alkali and water, and the finished solution can at once be used for nesslerizations.

Preparation of the double iodide as described above is not only less expensive than when mercuric iodide is employed, but it also avoids the impurities often found in the latter.

From a completely saturated sodium hydroxide solution, containing about 55 gm. of NaOH per 100 c.c., decant the clear supernatant liquid and dilute to a concentration of 10 per cent. (It is desirable to ascertain by titration that a 10 per cent solution has been obtained with an error of not over 5 per cent.) Introduce into a large bottle 3500 c.c. of 10 per cent sodium hydroxide solution, add 750 c.c. of the double iodide solution, and 750 c.c. of distilled water, giving 5 liters of Nessler's solution.

In the absence of modifying circumstances, such as the presence of much acid or alkali, this reagent should be added in the proportion of 10 c.c. per 100 c.c. of the volume to which the nesslerized solution is to be diluted. As a general rule the graduated flask, cylinder or test tube should be at least two-thirds full before adding the Nessler reagent. If attention is not given to this detail, turbid mixtures are obtained, and turbid solutions must never be used for color comparisons.

Nitrogen Solution, Standard.—A standard solution containing 1 mg. of N per 5 c.c. of solution may be prepared by dissolving 0.944 gm. ammonium sulfate or 0.764 gm. ammonium chloride of the highest purity in distilled water and making up to 1000 c.c.

The weaker solution for use with the test tube colorimeter is made by diluting this 1 to 10.

For the Folin-Wu methods the standard is diluted to contain 1 mg. of N per 10 c.c. of solution.

Paraffin and Vaseline Mixture.—Equal parts melted together as lubricant for stopcocks in Van Slyke CO_2 apparatus.

Permutit.—For estimation of ammonia in urine.

Phenolphthalein.—One per cent alcoholic solution for use with Van Slyke CO_2 apparatus.

Phenolsulfonephthalein Standard.—Ampoules for injection and preparation of standard.

To prepare the standard, break open one ampoule of the 'phthalein, pipette up 1 c.c. with an Ostwald-Folin pipette, and discharge into a two liter volumetric flask. Now add one liter of distilled water, and then sufficient strong sodium hydroxide solution to bring out the maximum color with the 'phthalein, and finally make up to the two liter mark with distilled water. Since the 1 c.c. from the ampoule contains 6 mg. of the drug, the solution now contains 3 mg. to the liter.

Phenol Red Solution (for pH determination in blood plasma).—This is prepared according to Clark by grinding 0.1 gm. of phenol red (phenolsulfonephthalein) in an agate mortar with 5.7 c.c. of 0.05 N NaOH and making up to 25c.c. The 0.4 per cent solution is then diluted as needed to the 0.02 per cent strength with distilled water.

Phosphate Solutions, Sörensen's Buffer (employed as standards in pH determination in blood plasma).—Satisfactory M/15 phosphate solutions may be prepared from Merck's special reagent chemicals.

The M/15 primary (acid) potassium phosphate, KH_2PO_4, is prepared by dissolving 9.08 gm. of the salt in freshly distilled, CO_2-free, water and making up to 1 liter. This solution will keep for some weeks in Pyrex glass in the refrigerator.

The M/15 secondary (alkaline) sodium phosphate, anhydrous Na_2HPO_4, is similarly prepared by dissolving 9.47 gm. of the anhydrous salt in water and making up to 1 liter. The solution is similarly preserved.

Phosphate Standard (for phosphorus in blood).—A standard solution containing 0.025 mg. of P per 3 c.c. of solution may be prepared by dissolving 0.1100 gm. of highest purity dry KH_2PO_4 in water and making up to 300 c.c. It should be preserved with chloroform.

Phosphomolybdic Acid Reagent (Folin-Wu).—Transfer to a liter beaker 35 gm. of molybdic acid and 5 gm. of sodium tungstate. Add 200 c.c. of 10 per cent sodium hydroxide and 200 c.c. of water. Boil vigorously for 20 to 40 minutes so as to remove nearly the whole of the ammonia present in the molybdic acid. Cool, dilute to about 350 c.c., and add 125 c.c. of concentrated (85 per cent) phosphoric acid. Dilute to 500 c.c.

Picramic Acid Standards (Benedict's clinical quantitative test for sugar in urine).—A 0.2 per cent solution of picramic acid in 0.5 per cent sodium carbonate, containing 30 c.c. of saturated picric acid per liter (previously made slightly alkaline or neutral with sodium carbonate) is prepared and diluted so that the color corresponds with that yielded by 0.1, 0.2, 0.3, 0.4 and 0.5 per cent solutions of glucose when treated exactly as described for urine and diluted to 22 c.c.

Picric Acid, Half Saturated (for sugar in normal urine).—Prepared by diluting the saturated solution with an equal volume of distilled water.

Picric Acid, Dry.—As purchased picric acid contains 10 per cent of added water. By exposure to the air between large filter papers (best in a warm place) the water will disappear by evaporation. Dry picric acid may be prepared in this way. If the alkaline picrate formed from this acid gives too deep a color (see test on p. 75) it may be purified as described below:

(a) Benedict method: It is best to start with the "technical" grade of commercial picric acid, and there is no need for preliminary removal of the 10 per cent of water which such samples contain. Four hundred gm. of the moist picric acid are placed in a 2 liter Pyrex flask and 1 liter of pure benzene is added. The mixture is heated to vigorous boiling on an electric plate with

occasional shaking at first to avoid bumping. Soon after the boiling point is reached the picric acid practically wholly dissolves, leaving a residue of dirt and foreign material, together with the water, which settles quickly to the bottom when the mixture is not boiling. The hot mixture (as nearly boiling as possible) is poured upon a large fluted filter which has been previously moistened with benzene, the solution being poured slowly enough so that most of the foreign sediment, together with the water, remains in the flask. This material (about 50 c.c. in volume) should be discarded, and not poured upon the filter. The clear filtrate should be received in a beaker of about 2 liters capacity. After the filtration is completed (it should not require more than 10 to 15 minutes) the beaker is covered with a large watch-glass, and heated on the electric plate until the picric acid which has begun to crystallize is again brought into solution. The covered beaker is then allowed to stand at room temperature for several hours or preferably over night without agitation. After this period the picric acid will be found to have crystallized on the bottom and sides of the beaker in large hard yellow-brown crystals, from which the excess of benzene can readily be drained without recourse to any filtration. The crystalline mass is washed twice by gentle rotary shaking with 75 c.c. portions of benzene, and the residue finally allowed to drain thoroughly for 15 to 30 minutes. For the draining it is convenient to invert partially the beaker in a large glass funnel. The crystallized picric acid can now be freed from the last of the benzene by drying in the air (which requires a long time and is hence undesirable in the average laboratory on account of access of impurities) or better by placing in an air bath at about 80° for a few hours, with occasional stirring. The product should be finally powdered by gentle rubbing in a mortar, and preserved in a brown glass-stoppered bottle. About 85 per cent of the picric acid used is recovered in the purified form. The benzene may be recovered by distillation preferably *in vacuo* from a water bath.

(b) Folin and Doisy method: Transfer about 600 gm. of wet picric acid, or about a pound of dry picric acid, to a large beaker (capacity not less than 4 liters). Pour on boiling water until the beaker is nearly full and add 200 c.c. of saturated (50 per cent) sodium hydroxide solution. Stir, and if necessary heat again until all the picric acid has been dissolved, yielding a deep red picrate solution. To the hot solution add rather slowly, with stirring, 200 gm. of sodium chloride. Cool in running water to about 30°C., with occasional stirring. Filter on a large Buchner funnel and wash a few times with 5 per cent sodium chloride solution. Transfer the picrate to a large beaker, fill with boiling water, and when the picrate is dissolved add, with stirring, first 50 c.c. of 10 per cent sodium hydroxide solution, and then 100 gm. of sodium chloride. Cool to 30°C., with stirring, filter, and wash with sodium chloride solution, as before. Repeat the solution and precipitation of the sodium picrate once more, but for the last washing of the last precipitated picrate use distilled water instead of the sodium chloride solution.

Dissolve the purified picrate in the same large beaker with boiling distilled water, and filter while hot on a large folded filter, collecting the filtrate in a large flask. To the hot filtrate add 100 c.c. of concentrated sulfuric acid, previously diluted with about two volumes of water. The liberated picric acid begins to come out at once. Put a beaker over the mouth of the flask and cool under running tap water to about 30°C. Filter with suction as before and wash free from sulfates with distilled water.

Picric Acid, Saturated Solution (for creatinine and sugar).—This may be prepared either by allowing distilled water to stand in contact with an excess of picric acid with occasional shaking, or by making a 1.2 per cent solution.

Plaster of Paris.—For cholesterol estimation.

Potassium Bichromate (for creatinine in urine).—A 0.5 N solution of potassium bichromate, 24.55 gm. to 1000 c.c. of water, is used as a color standard in estimating creatinine in urine.

Potassium Cyanide (for uric acid).—5 per cent solution; deteriorates on long standing.

Potassium Ferricyanide (for oxygen in blood).—A 20 per cent aqueous solution.

Potassium Oxalate.—A 20 per cent solution for use in the taking of blood to prevent clotting.

0.01 N Potassium Permanganate (for calcium in blood).—It has been our custom to prepare this from stock 0.1 N solution by a 1 to 10 dilution, checking each preparation against 0.01 N oxalic acid solution. A very satisfactory oxalic acid standard may be prepared from ammonium oxalate. This is an anhydrous salt and may be obtained perfectly pure. To make a 0.1 N solution of oxalic acid weigh up 6.2045 gm. of ammonium oxalate, dissolve in about 500 c.c. of water, add 50 c.c. of pure conc. sulfuric acid, and make up to 1 liter. This is diluted to the 0.01 N strength as needed. To prepare the 0.1 N potassium permanganate, dissolve 3.162 gm. (or 3.2 gm. quite as well) of potassium permanganate in about 900 c.c. of distilled water and allow to stand for a few days, then filter through glass wool. Check against the 0.1 N oxalic acid and dilute to volume. The 0.01 N potassium permanganate is standardized using the same apparatus as employed for the blood determination. Small portions of the 0.01 N oxalic acid (5 to 10 c.c.) are measured into the centrifuge tubes, subsequently to be used for the blood determination, heated to 75° C., and titrated, as a check on the standardization.

Clark points out that in the titration of calcium oxalate obtained by direct precipitation the end-point is not as permanent as in the case of pure inorganic solutions and the amount of permanganate necessary for the blank is greater (especially true with whole blood). The presence of traces of organic matter accounts for the above conditions and in order to decide upon a reproducible end-point it is advisable for each operator to make a series of preliminary determinations. The blanks are determined from samples of whole blood and plasma which have been treated according to the reg-

ular procedures except that no oxalate is added. Clark found the following averages for blank determinations: (1) whole blood = 0.10 c.c. of 0.01 N potassium permanganate; (2) plasma = 0.08 c.c. of 0.01 N potassium permanganate; and (3) sulfuric acid (5 c.c. of N) = 0.05 c.c. of 0.01 N potassium permanganate.

Potassium Persulfate Solution (Wong's oxidizing agent in micro N determination).—A saturated solution is employed. This may be prepared by shaking 7 gm. potassium persulfate with 100 c.c. of water in a glass stoppered bottle. The undissolved part settles on the bottom and keeps the solution saturated even if there be slight decomposition.

Potassium Sulfate (for nitrogen).—Powder; the potassium sulfate raises the boiling point of the sulfuric acid in the micro-Kjeldahl digestion.

Protein Standard (Folin's clinical quantitative test for protein in urine).—A stock protein solution of approximately 1 per cent strength is made by diluting sheep serum with 7 volumes of 15 per cent sodium chloride solution. This keeps well and the 0.1, 0.2, 0.3 and 0.4 per cent standards are made from it by dilution with 15 per cent sodium chloride solution.

Reagents for Determination of Total Acetone Bodies (Van Slyke).—Solutions required for the determination of total acetone bodies are:

20 per cent Copper Sulfate.—200 gm. of $CuSO_4.5H_2O$ dissolved in water and made up to 1 liter.

10 per cent Mercuric Sulfate.—73 gm. of pure red mercuric oxide dissolved in 1 liter of H_2SO_4 of 4 N concentration.

50 Volumes per cent Sulfuric Acid.—500 c.c. of sulfuric acid of 1.835 specific gravity, diluted to 1 liter with water. Concentration of H_2SO_4 must be readjusted if necessary to make it 17.0 N by titration.

10 per cent Calcium Hydroxide Suspension.—Mix 100 gm. of Merck's fine light "reagent" $Ca(OH)_2$ with 1 liter of water.

5 per cent Potassium Dichromate.—50 gm. $K_2Cr_2O_7$ dissolved in water and made up to 1 liter.

Combined Reagents for Total Acetone Bodies Determination.—1 liter of the above 50 per cent sulfuric acid, 3.5 liters of the mercuric sulfate, 10 liters of water.

Saline Solution (for pH determination in blood plasma).—A 0.9 per cent solution of sodium chloride in CO_2-free water, to which has been added 10 c.c. of 0.02 per cent phenol red solution for each 100 c.c., is adjusted to a pH of between 7.4 and 7.5 with sodium hydroxide. This can conveniently be done by dipping the tip of a long slender stirring rod in 1 per cent sodium hydroxide, stirring into the solution in the flask, and repeating the operation until the solution, when placed in the cup of the colorimeter, gives the correct reading. A method of preserving this solution is described in the text (see p. 118).

The phenol red solution above is prepared as follows: 0.1 gm. of phenol red is ground in an agate mortar with 5.7 c.c. of 0.05 N NaOH and made up

to 25 c.c. The 0.4 per cent solution is then diluted as needed to the 0.02 per cent strength with distilled water.

Saponin (for oxygen).—1 per cent solution of Merck's saponin.

Silver Lactate Solution (Folin's for uric acid estimation).—Dissolve 100 gm. of silver lactate in about 700 c.c. of warm water. To 100 c.c. of 85 per cent lactic acid add 100 c.c. of 10 per cent sodium hydroxide. Pour this partly neutralized lactic acid into the silver lactate solution, dilute to 1 liter, and set aside to allow the sediment, always present, to settle. Use only the clear supernatant solution. By thus neutralizing a part of the lactic acid, one provides for any excess acidity present in the blood filtrate and also for traces of mineral acids which may be present in some samples of silver lactate or in the lactic acid.

Silver Nitrate Standard (for chlorides in blood and urine).—See below and also chloride standards.

Silver Nitrate Solution, Standard (M/35.46) (for blood chlorides according to Whitehorn).—Dissolve 4.791 gm. of C. P. silver nitrate in distilled water. Transfer this solution to a liter volumetric flask and make up to the mark with distilled water. Mix thoroughly and preserve in a brown bottle. 1 c.c. = 1 mg. Cl. (It is to be noted that the silver nitrate and nitric acid are not added to the protein free filtrate simultaneously. To do so may result in the mechanical enclosure of silver nitrate solution within the curds, and a consequent error in the positive direction.)

Sodium Carbonate (for sugar in urine).—Anhydrous powder.

Sodium Carbonate (for urea, sugar and uric acid).—A saturated or 22 per cent solution.

Sodium Carbonate Solution (for Folin amino acid determination).—The required carbonate solution is made as follows: 50 c.c. of approximately saturated solution are diluted to a volume of 500 c.c. The strength of the resulting solution is determined by titrating 20 c.c. of 0.1 N hydrochloric acid with the carbonate, and with methyl red as indicator. On the basis of the titration value thus obtained, the carbonate solution is diluted so that 8.5 c.c. are equivalent to 20 c.c. of 0.1 N acid. The carbonate solution is about 1 per cent.

The correct degree of alkalinity is obtained when 1 c.c. of this sodium carbonate solution is added to 1 c.c. of amino acid soluton, which at the same time is a 0.1 N solution of hydrochloric acid. The alkalinity is, therefore, represented by a mixture of carbonate and bicarbonate. A drop of phenolphthalein should always be used when working with amino acids of unknown and variable acidity. It is advisable that the alkalinity in the different solutions, the standard and unknown, should be approximately the same, but there is no need of trying to make them exactly equal.

Sodium Chloride Solution (Folin's for uric acid estimation).—This solution contains 10 per cent of sodium chloride in 0.36 per cent hydrochloric acid. It may easily be prepared by adding 1 c.c. of pure concentrated hydro-

chloric acid (which should be 36-37 per cent) to 100 c.c. of 10 per cent sodium chloride solution.

Sodium Cyanide Solution (Benedict's, for uric acid in blood).—A 5 per cent solution of sodium cyanide, containing 2 c.c. of concentrated ammonia per liter.

Sodium Cyanide Solution (Benedict's, for uric acid in urine).—A 5 per cent solution of sodium cyanide, which should be prepared fresh once in about 2 months.

Sodium Cyanide (Folin's for uric acid determination).—This is a 15 per cent solution of sodium cyanide in 0.1 N sodium hydroxide. The sodium hydroxide is used simply to increase the keeping quality of the cyanide.

Sodium Hydroxide (for creatinine and 'phthalein).—A 10 per cent solution.

Sodium Hydroxide (for sugar in normal urine).—A 5 per cent solution.

Sodium Hydroxide (for nitrogen).—A saturated solution.

Sodium Hydroxide (for oxygen in blood).—A 0.5 N solution of NaOH (approximately 2 per cent).

Sodium Sulfate Solution (for urea in saliva).—A 20 per cent solution of sodium sulfate is used.

Sodium Thiosulfate Solution ($Na_2S_2O_3.5H_2O$) (for amino acid N determination).—A 4 per cent solution of sodium thiosulfate is used to destroy the surplus quinone remaining after the full color obtainable from the amino acids has developed. It destroys the surplus color of the quinone and under the conditions prescribed has no effect on the colored quinone-amino acid derivative, at least during the first 1 or 2 hours.

Sodium Tungstate (for Folin-Wu protein precipitation).—A 10 per cent solution is employed. Not all brands of sodium tungstate are suitable. The sodium tungstate must be very easily soluble in cold water. If it is not easily soluble, its solutions are not alkaline to phenolphthalein, and this shows that the product consists of complex tungstates (paratungstates). Folin states that such tungstates can be rendered serviceable as follows: Prepare a hot 10 per cent solution. Allow it to cool. Titrate 25 c.c. of the solution with 10 per cent sodium hydroxide, using phenolphthalein as indicator. The right end point is obtained only when the pink color produced by the alkali remains for at least 3 minutes. The titration figure so obtained will serve as a basis for calculating how much sodium hydroxide is required for 100 gm. of sodium tungstate in order to convert the paratungstate into the true simple tungstate. Add the calculated amount, and heat until solution is obtained.

For large scale work it is advantageous to prepare two liters or more of 20 per cent sodium tungstate solution which is weakly alkaline to phenolphthalein. From this stock solution the calcium will gradually settle out, and the clear supernatant solution can then be used as desired for the preparation of the Folin-Denis uric acid reagent or (after diluting with an equal volume of water) as the blood protein precipitant.

Soluble Starch (for diastatic activity).—A 1 per cent solution of soluble starch. Starch containing much in excess of 6 per cent reducing sugar is not suitable for this work. Small (Jour. Am. Chem. Soc., 1919, xli, 113) has described a method of preparing soluble starch from potato starch which is practically free from reducing sugar.

Soluble Starch (Small's method of preparation).—The method is as follows: (a) Preparation of potato starch.—Carefully washed and peeled potatoes are grated to a pulp; the starch is separated from the bulk of the fiber by leaching it out through several layers of cheesecloth, using large volumes of water and actively manipulating the starch-bearing pulp within the cheesecloth bag. The starch is separated from the small amount of fiber passing through the bag, by means of sedimentation. Continued washing by decantation frees the starch granules from soluble impurities. The washed starch is then dried in the air at room temperature and put through a 100-mesh sieve which removes the last traces of fiber.

(b) Preparation of soluble starch from potato starch.—Twenty gm. potato starch are transferred to an Erlenmeyer flask. (Arrangements should previously have been made to connect the flask with a reflux condenser and place on a boiling water bath.) The starch is now suspended in 100 c.c. of redistilled 95 per cent alcohol and 0.75 c.c. of conc. hydrochloric acid (sp. gr. 1.19) is added. The flask is now placed in the boiling water bath for exactly 10 minutes, and vigorously shaken from time to time to keep the starch from settling out. This should give a 100 per cent conversion of starch to soluble starch.

At the end of the 10 minutes, the flask is removed and immediately filtered, the soluble starch being washed with a large volume of distilled water until acid free, or the acid is immediately neutralized with the exact amount (previously ascertained by titration) of a solution of sodium bicarbonate, added in mass. In the latter case the alcohol is first decanted off, then fresh alcohol added and the starch transferred to a Buchner funnel, again washed, dried, sieved and preserved.

Sulfuric Acid, Concentrated (for nitrogen and cholesterol).—For the non-protein nitrogen and total nitrogen determinations, special, nitrogen free, sulfuric acid should be used.

Sulfuric Acid, Approximately Normal (for calcium estimation).—This may be prepared by adding 28 c.c. of pure conc. sulfuric acid to 970 c.c. of distilled water.

Sulfuric Acid ⅔ N (for Folin-Wu protein precipitation).—A ⅔ normal sulfuric acid solution is employed. This may be prepared by adding 35 gm. c. p. sulfuric acid to about 700 c.c. of water in a liter volumetric flask, cooling, then making up to volume. Although this solution will usually be found correct, it is necessary to check it up by titration. The ⅔ normal sulfuric acid is intended to be equivalent to the sodium content of the tungstate, so that when equal volumes are mixed, practically all the tungstic acid is set free without the presence of an excess of sulfuric acid. The tungstic acid set

free is nearly all taken up by the proteins, and the blood filtrates obtained are therefore only slightly acid to congo red paper.

Sulfuric Acid (for CO_2).—A 5 per cent solution.

Sulfocyanate Solution, Standard (M/35.46) (for blood chlorides according to Whitehorn).—Because sulfocyanates are hygroscopic, the standard solution must be prepared volumetrically. As an approximation about 3 gm. of potassium sulfocyanate or 2.5 gm. of ammonium sulfocyanate are dissolved in a liter of water. After ascertaining the strength of the sulfocyanate solution by titrating under the conditions described in the method, one may dilute the sulfocyanate so that 5 c.c. are equivalent to 5 c.c. of the silver nitrate solution.

Trichloracetic Acid.—A 2.5 or 5 per cent solution of trichloracetic acid is employed in the estimation of the nonprotein nitrogen and a 20 per cent solution in the estimation of the inorganic phosphorus.

Urease.—Concentrated preparations of jack bean urease may be obtained on the market (Arlington, Squibb), prepared according to the Van Slyke formula, i.e., containing the activating phosphate. Folin has pointed out that the keeping power of these preparations is not constant and recommends the procedure below:

Urease Extract (Folin).—Wash about 3 gm. of permutit in a flask once with 2 per cent acetic acid, then twice with water; add 5 gm. of fine jack bean meal (may be obtained from Arlington Chemical Co.) and 100 c.c. of 15 per cent alcohol (16 c.c. of ordinary alcohol plus 84 c.c. of water). Shake gently but continuously for 10 to 15 minutes, pour on a large filter and cover with a watch glass. The filtrate contains practically the whole of the urease and extremely little of other materials. The urease solution will keep for about a week at room temperature and for 4 to 6 weeks in an ice box.

Urease Paper (Folin).—Shake 30 gm. of jack bean powder with 10 gm. of permutit and 20 c.c. of 16 per cent alcohol for 10 to 15 minutes. Pour on one or two filters, and as soon as the filtration is substantially finished, pour the filtrate into a clean flat-bottomed dish. Draw strips of filter paper through the solution and hang them up to dry. They dry very quickly, and, once dry, the urease seems to keep just as well as it does in the original jack bean powder. Half a square inch of such a paper is enough for each blood urea determination.

Uric Acid Reagent, Benedict's New (for uric acid in blood and urine).—The reagent employed is the one used in the new procedure for the direct determination of uric acid in blood and urine, and is prepared as follows: 100 gm. of pure sodium tungstate are placed in a liter Pyrex flask and dissolved in about 600 c.c. of water. 50 gm. of pure arsenic acid (As_2O_5) are now added, followed by 25 c.c. of 85 per cent phosphoric acid and 20 c.c. of concentrated hydrochloric acid. The mixture is boiled for about 20 minutes, cooled, and diluted to 1 liter. The reagent appears to keep indefinitely.

Uric Acid Reagent (Folin-Denis).—Transfer 100 gm. of sodium tungstate to a 2 liter flask and add 750 c.c. of distilled water. Shake until solution is

obtained. A little white, fine, insoluble residue remains, due to presence of calcium. To the solution add 80 c.c. of 85 per cent phosphoric acid, ordinary phosphoric acid syrup. Close the mouth of the flask with a funnel plus two watch glasses, one small and one large, and boil gently but continuously for 2 hours. The color of the solution depends upon how much organic material is present. If it is very dark it may be bleached by the addition of a few drops of bromine, but this is usually superfluous. Boil for 10 to 15 minutes more to remove the surplus bromine. Cool and dilute to 1 liter.

Uric Acid Standard, Benedict's Dilute (for uric acid in blood and urine). —A standard solution of uric acid, acidified with hydrochloric acid, containing 0.2 mg. of uric acid in 10 c.c., is employed. This solution may be readily prepared by dilution of the phosphate standard uric acid solution of Benedict, as follows: 50 c.c. of the phosphate standard solution (containing 10 mg. of uric acid) are measured into a 500 c.c. volumetric flask and diluted to about 400 c.c. with distilled water. 25 c.c. of dilute hydrochloric acid (made by diluting 1 volume of the concentrated acid with 9 volumes of water) are added, and the solution is diluted to 500 c.c. and mixed. This dilute standard solution should be prepared fresh from the phosphate standard every 10 days to 2 weeks.

Benedict's Phosphate Standard is prepared as follows: Dissolve 4.5 gm. of pure crystalline hydrogen disodium phosphate and 0.5 gm. of dihydrogen sodium phosphate in about 200 c.c. of hot water. Pour this warm, clear solution on 100 mg. of uric acid suspended in a few c.c. of water in a 500 c.c. beaker. Agitate with a stirring rod until the uric acid is *completely* dissolved. Now add at once exactly 0.7 c.c. glacial acetic acid. Pour into a 500 c.c. volumetric flask, cool, make up to 500 c.c. mark with distilled water, mix and add 5 c.c. of chloroform as preservative. Five c.c. of this solution contain 1 mg. of uric acid. The solution should be freshly prepared every 4 to 8 weeks.

Uric Acid Standard, Folin's.—Transfer 100 mg. of uric acid to a clean 100 c.c. beaker. Dissolve 50 mg. of lithium carbonate in 20 c.c. of water in a large test tube or small beaker and heat to 60 to 70° C., and pour this warm alkali on the uric acid and stir until the uric acid is completely dissolved. As soon as a clear solution is obtained, add 5 c.c. of 40 per cent formaldehyde and then 1 c.c. of 50 per cent acetic acid. Transfer the solution quantitatively to a 100 c.c. volumetric flask and dilute to volume, mix and transfer to a bottle. This solution contains 1 mg. of uric acid per cubic centimeter.

To prepare the dilute standard for blood analysis, transfer 1 c.c. containing 1 mg. of uric acid, to a 250 c.c. volumetric flask. Half fill the flask with water; then add 10 c.c. of two-thirds normal sulfuric acid, and 1 c.c. (but no more) of 40 per cent formaldehyde. Dilute to the mark with water and mix. This solution should keep for at least a month. Use 5 c.c., containing 0.02 mg. of uric acid for each determination.

FOUR PLACE

NOS.	0	1	2	3	4	5	6	7	8	9	PROPORTIONAL PARTS								
											1	2	3	4	5	6	7	8	9
10	0000	0043	0086	0128	0170	0212	0253	0294	0334	0374	4	8	12	17	21	25	29	33	37
11	0414	0453	0492	0531	0569	0607	0645	0682	0719	0755	4	8	11	15	19	23	26	30	34
12	0792	0828	0864	0899	0934	0969	1004	1038	1072	1106	3	7	10	14	17	21	24	28	31
13	1139	1173	1206	1239	1271	1303	1335	1367	1399	1430	3	6	10	13	16	19	23	26	29
14	1461	1492	1523	1553	1584	1614	1644	1673	1703	1732	3	6	9	12	15	18	21	24	27
15	1761	1790	1818	1847	1875	1903	1931	1959	1987	2014	3	6	8	11	14	17	20	22	25
16	2041	2068	2095	2122	2148	2175	2201	2227	2253	2279	3	5	8	11	13	16	18	21	24
17	2304	2330	2355	2380	2405	2430	2455	2480	2504	2529	2	5	7	10	12	15	17	20	22
18	2553	2577	2601	2625	2648	2672	2695	2718	2742	2765	2	5	7	9	12	14	16	19	21
19	2788	2810	2833	2856	2878	2900	2923	2945	2967	2989	2	4	7	9	11	13	16	18	20
20	3010	3032	3054	3075	3096	3118	3139	3160	3181	3201	2	4	6	8	11	13	15	17	19
21	3222	3243	3263	3284	3304	3324	3345	3365	3385	3404	2	4	6	8	10	12	14	16	18
22	3424	3444	3464	3483	3502	3522	3541	3560	3579	3598	2	4	6	8	10	12	14	15	17
23	3617	3636	3655	3674	3692	3711	3729	3747	3766	3784	2	4	6	7	9	11	13	15	17
24	3802	3820	3838	3856	3874	3892	3909	3927	3945	3962	2	4	5	7	9	11	12	14	16
25	3979	3997	4014	4031	4048	4065	4082	4099	4116	4133	2	3	5	7	9	10	12	14	15
26	4150	4166	4183	4200	4216	4232	4249	4265	4281	4298	2	3	5	7	8	10	11	13	15
27	4314	4330	4346	4362	4378	4393	4409	4425	4440	4456	2	3	5	6	8	9	11	13	14
28	4472	4487	4502	4518	4533	4548	4564	4579	4594	4609	2	3	5	6	8	9	11	12	14
29	4624	4639	4654	4669	4683	4698	4713	4728	4742	4757	1	3	4	6	7	9	10	12	13
30	4771	4786	4800	4814	4629	4843	4857	4871	4886	4900	1	3	4	6	7	9	10	11	13
31	4914	4928	4942	4955	4969	4983	4997	5011	5024	5038	1	3	4	6	7	8	10	11	12
32	5051	5065	5079	5092	5105	5119	5132	5145	5159	5172	1	3	4	5	7	8	9	11	12
33	5185	5198	5211	5224	5237	5250	5263	5276	5289	5302	1	3	4	5	6	8	9	10	12
34	5315	5328	5340	5353	5366	5378	5391	5403	5416	5428	1	3	4	5	6	8	9	10	11
35	5441	5453	5465	5478	5490	5502	5514	5527	5539	5551	1	2	4	5	6	7	9	10	11
36	5563	5575	5587	5599	5611	5623	5635	5647	5658	5670	1	2	4	5	6	7	8	10	11
37	5682	5694	5705	5717	5729	5740	5752	5763	5775	5786	1	2	3	5	6	7	8	9	10
38	5798	5809	5821	5832	5843	5855	5866	5877	5888	5899	1	2	3	5	6	7	8	9	10
39	5911	5922	5933	5944	5955	5966	5977	5988	5999	6010	1	2	3	4	6	7	8	9	10
40	6021	6031	6042	6053	6064	6075	6085	6096	6107	6117	1	2	3	4	5	6	8	9	10
41	6128	6138	6149	6160	6170	6180	6191	6201	6212	6222	1	2	3	4	5	6	7	8	9
42	6232	6243	6253	6263	6274	6284	6294	6304	6314	6325	1	2	3	4	5	6	7	8	9
43	6335	6345	6355	6365	6375	6385	6395	6405	6415	6425	1	2	3	4	5	6	7	8	9
44	6435	6444	6454	6464	6474	6484	6493	6503	6513	6522	1	2	3	4	5	6	7	8	9
45	6532	6542	6551	6561	6571	6580	6590	6599	6609	6618	1	2	3	4	5	6	7	8	9
46	6628	6637	6646	6656	6665	6675	6684	6693	6702	6712	1	2	3	4	5	6	7	7	8
47	6721	6730	6739	6749	6758	6767	6776	6785	6794	6803	1	2	3	4	5	5	6	7	8
48	6812	6821	6830	6839	6848	6857	6866	6875	6884	6893	1	2	3	4	4	5	6	7	8
49	6902	6911	6920	6928	6937	6946	6955	6964	6972	6981	1	2	3	4	4	5	6	7	8
50	6990	6998	7007	7016	7024	7033	7042	7050	7059	7067	1	2	3	3	4	5	6	7	8
51	7076	7084	7093	7101	7110	7118	7126	7135	7143	7152	1	2	3	3	4	5	6	7	8
52	7160	7168	7177	7185	7193	7202	7210	7218	7226	7235	1	2	2	3	4	5	6	7	7
53	7243	7251	7259	7267	7275	7284	7292	7300	7308	7316	1	2	2	3	4	5	6	6	7
54	7324	7332	7340	7348	7356	7364	7372	7380	7388	7396	1	2	2	3	4	5	6	6	7

LOGARITHMS

NOS.	0	1	2	3	4	5	6	7	8	9	PROPORTIONAL PARTS								
											1	2	3	4	5	6	7	8	9
55	7404	7412	7419	7427	7435	7443	7451	7459	7466	7474	1	2	2	3	4	5	5	6	7
56	7482	7490	7497	7505	7513	7520	7528	7536	7543	7551	1	2	2	3	4	5	5	6	7
57	7559	7566	7574	7582	7589	7597	7604	7612	7619	7627	1	2	2	3	4	5	5	6	7
58	7634	7642	7649	7657	7664	7672	7679	7686	7694	7701	1	1	2	3	4	4	5	6	7
59	7709	7716	7723	7731	7738	7745	7752	7760	7767	7774	1	1	2	3	4	4	5	6	7
60	7782	7789	7796	7803	7810	7818	7825	7832	7839	7846	1	1	2	3	4	4	5	6	6
61	7853	7860	7868	7875	7882	7889	7896	7903	7910	7917	1	1	2	3	4	4	5	6	6
62	7924	7931	7938	7945	7952	7959	7966	7973	7980	7987	1	1	2	3	3	4	5	6	6
63	7993	8000	8007	8014	8021	8028	8035	8041	8048	8055	1	1	2	3	3	4	5	5	6
64	8062	8069	8075	8082	8089	8096	8102	8109	8116	8122	1	1	2	3	3	4	5	5	6
65	8129	8136	8142	8149	8156	8162	8169	8176	8182	8189	1	1	2	3	3	4	5	5	6
66	8195	8202	8209	8215	8222	8228	8235	8241	8248	8254	1	1	2	3	3	4	5	5	6
67	8261	8267	8274	8280	8287	8293	8299	8306	8312	8319	1	1	2	3	3	4	5	5	6
68	8325	8331	8338	8344	8351	8357	8363	8370	8376	8382	1	1	2	3	3	4	4	5	6
69	8388	8395	8401	8407	8014	8420	8426	8432	8439	8445	1	1	2	2	3	4	4	5	6
70	8451	8457	8463	8470	8476	8482	8488	8494	8500	8506	1	1	2	2	3	4	4	5	6
71	8513	8519	8525	8531	8537	8543	8549	8555	8561	8567	1	1	2	2	3	4	4	5	5
72	8573	8579	8585	8591	8597	8603	8609	8615	8621	8627	1	1	2	2	3	4	4	5	5
73	8633	8639	8645	8651	8657	8663	8669	8675	8681	8686	1	1	2	2	3	4	4	5	5
74	8692	8698	8704	8710	8716	8722	8727	8733	8739	8745	1	1	2	2	3	4	4	5	5
75	8751	8756	8762	8768	8774	8779	8785	8791	8797	8802	1	1	2	2	3	3	4	5	5
76	8808	8814	8820	8825	8831	8837	8842	8848	8854	8859	1	1	2	2	3	3	4	5	5
77	8865	8871	8876	8882	8887	8893	8899	8904	8910	8915	1	1	2	2	3	3	4	4	5
78	8921	8927	8932	8938	8943	8949	8954	8960	8965	8971	1	1	2	2	3	3	4	4	5
79	8976	8982	8987	8993	8998	9004	9009	9015	9020	9025	1	1	2	2	3	3	4	4	5
80	9031	9036	9042	9047	9053	9058	9063	9069	9074	9079	1	1	2	2	3	3	4	4	5
81	9085	9090	9096	9101	9106	9112	9117	9122	9128	9133	1	1	2	2	3	3	4	4	5
82	9138	9143	9149	9154	9159	9165	9170	9175	9180	9186	1	1	2	2	3	3	4	4	5
83	9191	9196	9201	9206	9212	9217	9222	9227	9232	9238	1	1	2	2	3	3	4	4	5
84	9243	9248	9253	9258	9263	9269	9274	9279	9284	9289	1	1	2	2	3	3	4	4	5
85	9294	9299	9304	9309	9315	9320	9325	9330	9335	9340	1	1	2	2	3	3	4	4	5
86	9345	9350	9355	9360	9365	9370	9375	9380	9385	9390	1	1	2	2	3	3	4	4	5
87	9395	9400	9405	9410	9415	9420	9425	9430	9435	9440	0	1	1	2	2	3	3	4	4
88	9445	9450	9455	9460	9465	9469	9474	9479	9484	9489	0	1	1	2	2	3	3	4	4
89	9494	9499	9504	9509	9513	9518	9523	9528	9533	9538	0	1	1	2	2	3	3	4	4
90	9542	9547	9552	9557	9562	9566	9571	9576	9581	9586	0	1	1	2	2	3	3	4	4
91	9590	9595	9600	9605	9609	9614	9619	9624	9628	9633	0	1	1	2	2	3	3	4	4
92	9638	9643	9647	9652	9657	9661	9666	9671	9675	9680	0	1	1	2	2	3	3	4	4
93	9685	9689	9694	9699	9703	9708	9713	9717	9722	9727	0	1	1	2	2	3	3	4	4
94	9731	9736	9741	9745	9750	9754	9759	9763	9768	9773	0	1	1	2	2	3	3	4	4
95	9777	9782	9786	9791	9795	9800	9805	9809	9814	9818	0	1	1	2	2	3	3	4	4
96	9823	9827	9832	9836	9841	9845	9850	9854	9859	9863	0	1	1	2	2	3	3	4	4
97	9868	9872	9877	9881	9886	9890	9894	9899	9903	9908	0	1	1	2	2	3	3	4	4
98	9912	9917	9921	9926	9930	9934	9939	9943	9948	9952	0	1	1	2	2	3	3	4	4
99	9956	9961	9965	9969	9974	9978	9983	9987	9991	9996	0	1	1	2	2	3	3	3	4

International Atomic Weights, 1922

O = 16

Aluminium	Al	27.0	Manganese	Mn	54.93
Antimony	Sb	120.2	Mercury	Hg	200.6
Arsenic	As	74.96	Molybdenum	Mo	96 0
Barium	Ba	137.37	Nickel	Ni	58.68
Bismuth	Bi	209.0	Nitrogen	N	14.008
Boron	B	10.9	Osmium	Os	190.9
Bromine	Br	79.92	Oxygen	O	16.00
Cadmium	Cd	112.40	Palladium	Pd	106.7
Calcium	Ca	40.07	Phosphorus	P	31.04
Carbon	C	12.005	Platinum	Pt	195.2
Chlorine	Cl	35.46	Potassium	K	39.10
Chromium	Cr	52.0	Radium	Ra	226.0
Cobalt	Co	58.97	Selenium	Se	79.2
Copper	Cu	63.57	Silicon	Si	28.1
Fluorine	F	19.0	Silver	Ag	107.88
Glucinum	Gl	9.1	Sodium	Na	23.00
Gold	Au	197.2	Strontium	Sr	87.63
Hydrogen	H	1.008	Sulfur	S	32.06
Iodine	I	126.92	Tantalum	Ta	181.5
Iridium	Ir	193.1	Tellurium	Te	127.5
Iron	Fe	55.84	Tin	Sn	118.7
Lanthanum	La	139.0	Titanium	Ti	48.1
Lead	Pb	207.20	Tungsten	W	184.0
Lithium	Li	6.94	Uranium	U	238.2
Magnesium	Mg	24.32	Zinc	Zn	65.37

Metric Equivalents

1 inch	=	2.54	centimeters
1 foot	=	30.48	"
1 grain (Troy)	=	0.065	grams
1 scruple (Apothecaries')	=	1.295	"
1 dram (")	=	3.885	"
1 ounce (")	=	31.10	"
1 ounce (Avoirdupois)	=	28.35	"
1 pound (")	=	453.59	"
1 minim (Fluid Measure, Apothecaries')	=	0.06	c.c.
1 fluid ounce (" " ")	=	29.57 (30)	c.c.
1 pint (" " ")	=	473.11	"
1 quart (" " ")	=	946.22	"
1 gallon (" " ")	=	3785.00	"

1 centimeter	=	0.3937 inches
1 meter	=	39.3704 "
1 gram	=	15.4323 Troy grains
1 kilogram	=	2.205 Avoirdupois pounds
1 cubic centimeter	=	16.23 (16) minims
100 " "	=	3.38 fluid ounces
1 liter	=	33.81 " "

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